

Agilent Mass Profiler Software

Quick Start Guide

What is Mass Profiler Software?

Mass Profiler operates on the extracted data files (.mhd files) produced by Mass Hunter to let you investigate similarities and differences in features across multiple analyses/samples.

Comparing feature information

A feature is a discrete molecular entity defined by the combination of retention time and mass. The software aligns and normalizes features from different .mhd files generated by Mass Hunter. It presents the results in both graphic and tabular forms.

From the plot:

- Investigate features using three different plot types: Mass vs Retention Time, Log 2 Ratio vs Retention Time or Log/Log plot.
- Investigate features in all samples or in one sample
- Investigate only features above the visibility threshold
- Investigate features by color (color each feature a different color, or color all features in the same .mhd file the same color, or color all features in the same group the same color)

From the table:

- View all the features for their summary, group statistics and differences between the two groups.
- View individual feature details, including species clusters found in a selected .mhd file and possible compositions for the feature

You can also export most of the data generated by Mass Profiler to an .xls file or to a .txt file for import to GeneSpring.

For a complete list of tasks, see the Mass Profiler online help.



Getting started with the Mass Profiler software

Install the software

- 1 Go to the directory on the CD that contains the Mass Profiler setup.exe file.
- 2 Double-click setup.exe.
- **3** Follow the instructions on each screen of the InstallShield wizard, and click **Next** to move on to the next screen.

Accept the default path for the software.

4 Click **Finish** to complete the installation.

Start the software

• Double-click the Mass Profiler icon Δ_{λ} on the desktop, or

Select **Start > Programs > Agilent > Mass Profiler** from the desktop.

The system displays the Mass Profiler main window.

🔯 Mass Profiler
File Analysis Help

Learn how to access Mass Profiler functions

You can use the toolbar or the menus to prepare the data for comparison either before or after you load an .mhd folder:



Learn how to use Mass Profiler

Try these exercises to familiarize yourself with the Mass Profiler application. Try the **Steps** on the left in the exercises on the next pages without the **Detailed Instructions**. If you need more help, follow the detailed instructions.

If you want to do this:	Refer to this exercise:
Take a look at the all the features loaded with the default parameters	"Exercise 1 – Take a quick look at the results" on page 4
Filter, align, normalize or perform differential analysis on feature data	"Exercise 2 – Set up to process feature data" on page 7
Work with plot data to view the differences in features	"Exercise 3 – View Feature Plot" on page 10
Work with feature table data to view differences	"Exercise 4 – View Feature Table" on page 13
View feature species clusters or possible compositions	"Exercise 5 – View more detailed feature information" on page 15
Export Mass Profiler data to GeneSpring	"Exercise 6 – Export Mass Profiler Feature Summary for GeneSpring" on page 17

Exercise 1 – Take a quick look at the results

Before you start to change parameters on how to choose, process and display the data, you may want to take a quick look at the results with the default parameters..

Steps	Detailed Instructions	Comments			
 Load the example loadable folder, Example_Myoglobin_Spike_into_ BSA_mhd Copy this example Mass Profiler folder to a directory that only you will use. Mass Profiler lets you compare feature data in one set of .mhd files or in two sets, but not more. 	 a Select File > Load mhd folder. b Go to the directory that contains the Mass Profiler example folder. c Select the Example_Myoglobin_Spike_into _BSA_mhd folder. d Click OK. 741 average features (mass and RT) averaged over all 10 mhd files appear in the Feature Comparison Window in 3 plots and a table. 	 This loadable folder contains two groups of data. That is, it contains two subfolders, each with a different set of .mhd files. Group 1 – 5 .mhd files of 120 fmol Myoglobin in 60 fmol BSA digest. Group 2 – 5 .mhd files of 30 fmol Myoglobin in 60 fmol BSA digest. For an explanation of why log2 columns are in blue or red in the Feature Table, see Step 1 of Exercise 4 on page 13. 			





f Click the Log/Log Plot tab.



Steps			etailed Instructions	Comments			
3	Compare only features below this visibility threshold: • Visibility Criterion: max RSD abundance	1 2	As the Visibility Criterion, select max RSD abundance. Enter the Visibility Threshold as .09.	•	Note that the points along the 1X fold are BSA features and the points along the 4X are myoglobin. Note that one group of .mhd files		
•	• Visibility Threshold: .09.	nold: .09. The plot be at .09 thres Note that o max RSD al threshold a	The plot below shows the data points at .09 threshold.		contains 4X the concentration of myoglobin spike-in relative to the other group (120 vs 30).		
			Note that only those features whose max RSD abundance is below the threshold appear in the plot.	•	In another exercise, you will gather more information about one of these myoglobin features.		



Exercise 2 – Set up to process feature data

After an initial review of the data, you are now ready to change the default settings to meet your special needs. This exercise helps you prepare the .mhd files so that you can easily study features between different .mhd files.

Steps	Detailed Instructions	Comments
 Filter the features that are loaded into the application. Change these settings: RT – 12-34 min. 	 a Select Analysis > Filter Features, Click the Filter Features icon. A dialog box appears that is the sa one used for Display Filters in Mas Hunter. b For Min RT, enter 12. c For Max RT, enter 34. 	, or ame ss
	📕 Filter Features	X
	Use all the available data Min Max RT 12 34 min. Mass 0 100000 Da Isotope Pattern Formula Custom formula custom	Special Masses Exclude C Limit to these Tolerance 0.0050 Da Mass Defect (Da) Min -0.50 Max 0.50 Doubtril Features
		Feature w/o isotopes
	Charge State	features of unknown mass included excluded C limited to these
	Abundance Relative Absolut Min Relative Abu	e Top ndance 0.000 % Cancel

Steps	Detailed Instructions	Comments			
	d Click OK. The Feature Comparison Window now appears with only 602 average features.	 When you click OK, the settings are immediately applied, along with the current settings for aligning features and performing differential analysis. 			
 2 Change the alignment and normalization settings to these values: Use standards for RT correction and normalization. Use 3 internal standards with these RTs and masses: RT: 15.42; Mass: 921.4808 RT: 19.54; Mass: 1554.6298 RT: 28.21; Mass: 2458.1575 	 a Select Analysis > Align features, or Click the Align features icon. b For the RT correction method, select with standards. c For Normalization, select with standards. d From the No. of Internal Standards list, enter or select 3. e Make sure that the Use for RT correction and Use for normalization check boxes are marked. 				

angi reatures		A
Internal Standards	Alignment Parameters	Normalization
No. of Internal Standards 3	Mass tolerance	
BT(min) mass(Da) Use for Use for	Intercept 2.0 mDa	C Without Standards
RT correction normalization	Slope 5.0 ppm	
15.42 921.4808	RT tolerance	G Article Strandards
19.54 1554.6298	- Defere BT encodier	with standards
28.21 2458.1575 🔽 🔽	Intercept 0.5 min	
	Slope 0.5 %	C None
	RT correction method	
	C Without C With C None	
	After RT correction	
	Intercept 0.3 min	
	Slope 0.0 %	
	OK Cancel	
		11.

f Click OK.

The Feature Comparison Window now appears with only 529 features.

 When you click OK, the alignment settings are immediately applied to the feature plots and tables, as are the current filtering and differential analysis settings.

St	eps	D	etailed Instructions	Comments				
3	Change the differential analysis settings to these values: • Min log2(abund1/abund2) = 1	a b	Select Analysis > Differential Analysis, or Click the Differential Analysis icon. For Min log2(abund1/abund2) , enter 1.	•	Abund1 is the average abundance of a feature in the set1 .mhd files (120 fmol myo). Abund2 is the average abundance of a feature in the set2 .mhd files (30 fmol myo).			
			Differential Analysis		X			
			Analysis Preference		Result Filters			
			Missing-Info Treatment		Min differential core			
			O abundance C excluded		Min relative frequency			
			Differential Scoring		Group 1: 5/5 Group 2: 5/5 All: 10/10 C and unique to a single group			
			Student t-test		in at least one group			
					C of total			
			OK		Cancel			
		C	Click OK . The Feature Comparison Window now appears with only 265 average features.	•	When you click OK, the settings are immediately applied, along with the current settings for filtering and aligning/normalizing features.			

Exercise 3 – View Feature Plot

This exercise shows you how to view features using different plot functions.

Steps	Detailed Instructions	Comments			
 Compare the features in the log/log plot by color. Reset the Visibility Criterion to max RSD abundance and the threshold to 1.2. Select to have the features in each .mhd file in a different color. 	 a From the Visibility Criterion list, select max RSD abundance. b Enter a Visibility Threshold of 1.2. c From the Plot Style list, select colored by mhd file. Note that many more data points appear than were on the previous plot with Plot Style Avg. Feature 	 Avg. Feature is one data point—a result of averaging the masses and RTs of ten features over ten .mhd files. Data points for "Colored by mhd file" represent the same feature in each .mhd file, each of which has a different RT. 			





- **3** Compare the features in the Log2 Ratio vs Retention Time plot by color for all the .mhd files and find information on feature #222:
 - Color features by feature.
 - Color features by group.
 - Zoom into the RT range of 32.2-32.4.
 - Find information on mass, RT and abundance for feature #222 in the Feature Plot and Feature Table.

- a Click the Log2 Ratio vs RT tab.
- b From the Graph Data list, select All mhd files.
- c From the Plot Style list, select colored by feature.
- d From the Plot Style list, select colored by group.
- e Click a point around RT 32 that lets you include points between RT 32 and 34.
- f Draw a rectangle whose opposite corner is about RT 34 and release the mouse.
- **g** Continue to zoom in until you see RT 32.2 to 32.4 on the plot.
- h To find the feature with ID 222 on the plot, pass the cursor over the data points until you see the information in the tooltip for #222.
- i Click the #222 data point to now see that feature highlighted in the Feature Table.

- Note how many more colors are visible when you switch from "colored by mhd file" to "colored by feature".
- Note that when you select "colored by group", features are now colored by the group of .mhd files in which they are found.
- Group 1 = 120 fmol .mhd files = red Group 2 = 30 fmol .mhd files = blue



Exercise 4 – View Feature Table

You can also use the feature data in tabular form to investigate features across different .mhd files.

Steps				Detailed Instructions								Comments											
1 View the features in the Features Table for feature #222.								• Scroll the Features Table if necessary									The comparison data shows the actual differences in RT, mass, log						
 See the Reference Help for descriptions of the columns in the Feature Table. Export 265 Features 											• i (2 v	f the G s great 30-blu eature abund alue is	roup 1 (1 er than t e), the lo is showr dance is s shown i	20-red) a hat of Gro g2 ratio fo n in red. If greater, th n blue.	bundance oup 2 or the the Grou nen the	e p							
			Featu	re Su	ummaru		120	lfmol Mvo I	SOfmol B	SA(5)		30fi	nol Mvo	60fmol B	SA(5)		Compa	arison					
	ab	undance	•					abundanc	3				abundan	ce		Differer	ice				1		
	va	lue	R.S.D.	#	mark	RT	mass	value	R.S.D.	#	RT	mass	value	R.S.D.	#	RT	mass	log2(A1/A2)	log2(A1/A2)	Diff. Score			
	219	1.22	0.70	- 7		29.011	607.2612	0.64	1.37	2	28.995	607.2603	1.80	0.12	5	-0.016	-0.0009	-1.49	1.49	97.9			
	220	3.49	0.58	9		14.001	675.3779	1.86	0.58	4	13.998	675.3785	5.12	0.24	5	-0.003	0.0006	-1.46	1.46	99.8			
	221	0.54	0.99	6		17.239	1438.5914	0.78	0.33	5	17.249	1438.5852	0.29	2.24	1	0.010	-0.0062	1.45	1.45	85.2			
	222	235.41	0.49	10		32.314	731.4569	343.73	0.05	5	32.309	731.4569	127.08	0.11	5	-0.005	0.0000	1.44	1.44	100.0			
	223	0.92	0.70	- 7		26.447	1170.6077	1.35	0.12	5	26.444	1170.6041	0.50	1.38	2	-0.003	-0.0037	1.43	1.43	97.2			
	224	26.42	0.53	9		30.622	1411.6807	38.54	0.03	5	30.647	1411.6799	14.30	0.59	4	0.025	-0.0008	1.43	1.43	100.0			
	225	8.73	0.49	10		28.508	1442.6745	12.72	0.07	5	28.499	1442.6732	4.75	0.20	5	-0.009	-0.0013	1.42	1.42	100.0	 		
_																							

- the mass column and then by the ID.
- 2 Sort data by the RT column, then by a Double-click the RT column until you see the features in order of lowest RT to highest RT.
 - **b** Double-click the mass column header until you see the features in order of lowest mass to highest mass.
 - c Double-click the ID column to return the table to its original format.

Steps	Detailed Instructions	Comments								
3 View the abundance distribution graph for feature #222.	 a In the Features Table, right-click the row number for feature #222. b Select Abundance Distribution. 	 The Abundance Distribution window shows the abundance distribution of feature 222 in the two sets of .mhd files. 								
	Abundance Distribution of Feature # 222									
	120 180 240	300 360 abund.								
	4	2								
	10 12 14 16 18 20 22 24	26 28 30 32 34 36 38								
	x 1.0e+001									

4 Mark feature #222 for annotation.

 a In the Features Table, right-click feature #222, and select MarkOn/Off. You see an X in the Mark column next to feature #222.

Exp	Export 265 Features													
		Feature Summary												
		RT		mass		abundanc	е							
	ID	value	S.D.	value	S.D.	value	R.S.D.	#	mark					
219	219	28.999	0.017	607.2606	0.0016	1.22	0.70	7						
220	220	13.999	0.007	675.3782	0.0009	3.49	0.58	9						
221	221	17.240	0.011	1438.5904	0.0037	0.54	0.99	6						
222	222	32.312	0.008	731.4569	0.0002	235.41	0.49	10	X					
223	223	26.447	0.013	1170.6067	0.0025	0.92	0.70	- 7						
224	224	30.633	0.016	1411.6804	0.0012	26.42	0.53	9						
225	225	28.504	0.013	1442.6738	0.0030	8.73	0.49	10						

•

Exercise 5 – View more detailed feature information

This exercise shows you how to access information on species clusters and chemical compositions for individual features. You can see the species clusters for the feature in each of the .mhd files in which it is found and calculate the possible compositions for the feature.

Ste	eps	Detailed Inst	ructions	;	Co	omments			
1	Display the Feature Information Window for feature #222.	 In the Feat right-click Details (For A listing o .mhd files appears or 	tures Tal feature eature D f the fea contain n the lef	ble (or the plot), #222, and select l etails in the plot). ture data for all the ng the feature t.	•	A plot of the sp for the feature f appears on the	ecies clu for each right.	ıster d .mhd f	ata ïle
	🔜 Information for Feature # 222								
	Export All mhd files				120fmol_M	lyo_60fmol_BSA			<u> </u>
			200-		Nov2	9-71_1_1_1			
	ID Name RI mass 1 1 Nov29-71 1 32.303 731.456	abundance 8 371.16	200					1	
	2 2 Nov29-72_1_ 32.316 731.457	1 355.20	100	M+2H				M+H+1 M+H+2	
	3 3 Nov29-73_1_ 32.309 731.457 4 4 Nov29-74 1 32.319 731.456	3 334.75	300	400	500	600	700	•	800
	5 5 Nov29-75_1_ 32.322 731.456	7 328.01			Nov2	9-72_1_1_1			
	6 6 Nov29-61_1 32.325 731.457 7 7 Nov29-62 1 32.300 731.456	1 111.28	200					M+H	
	8 8 Nov29-63_1_ 32.305 731.456	9 129.27	100					#+H+1 #+H+2	
	9 9 Nov29-64_1_ 32.309 731.45t 10 10 Nov29-65 1 32.307 731.45t	8 128.70	0 +	400	500	600	700		
					Nov2	9-73_1_1			
)		300					M+H	
	Selected mhd file		100					H+H+1	
				400	500		700	M+n+2	800 -1
						00(1.004			
					3Ufmol_My	yo_6Umol_85A			^ _
			300 1		1092	3-01_1_1			
			200						
			0	M+2H			· · · · ·	####2	_
			300	400	500	600	700		800
			300		Nov2	¹ 9-62_1_1_1			
			200						
		Name a siti sa	100	M+2H				### #####2	
	Calculate	Joinposition	300	400	500	600	700		800
			300		Nov2	29-63_1_1_1			
			200						
			100	W+2H				III+H 数字符字2	
			300	400	500	600	700		800 -

Steps	Detailed Instructions	Comments
2 View the feature species table for .mhd file Nov29-72_1_1_1.	 Double-click the row number for .r file #2. The table listing the species cluste for feature #222 in that .mhd file appears. 	mhd ers
	Export Nov29-72_1_1_1	
		undance wid
	1 M 32.316 731.4571	355.20 0.
	2 M+2H 32.322 366.7367 731.4588	6.87 0.
	3 M+2H+1 32.314 367.2380	3.33 0.
	4 M+2H+2 32.296 367.7409	0.00
	6 M+H 32.320 732.4643 731.4570	224.22 0.
	7 M+H+1 32.320 733.4671	94.66 0.
	8 M+H+2 32.320 734.4701	21.24 0.
	10 M+H+4 32 321 736 4764	0.57 0
	11	
for feature #222.	s • Click Calculate .	 If you need to change the element selected to calculate the composition see the Mass Hunte
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. 	 If you need to change the element selected to calculate the composition, see the Mass Hunte Quick Start Guide.
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bol of the window. Export Chemistry Calculate Composition 	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Composition (chemical form dm(Da) dm(ppm) DBE score) 	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Composition of the chemical form dm(Da) dm(ppm) DBE see 1 C37H61N705 0.0014 1.9 11.0 	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bol of the window. Export Chemistry Calculate Composition of the window. Chemical form dm(Da) dm(ppm) DBE see 1 C37H61N706 0.0014 1.9 11.0 2 C37H53N230 0.0032 4.4 9.0 2 C31H53N230 0.0032 4.5 9.2 0 L2 C31H53N230 0.00100 0.00100 0.0010000000000000000	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bol of the window. Export Chemistry Calculate Composition of the window. Calculate Composition of the window. 	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Composition of the window. Calculate Composition of the window. 	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	 Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Composition of the window. Calculate Composition of the window. Calles Statistical of the window. 	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	s Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Composition of the window. Export Chemistry Calculate Composition of the window. Export Chemistry Calculate Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window.	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
for feature #222.	s Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Composition of the window. Export Chemistry Calculate Composition of the window. Export Chemistry Calculate Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Composition of the window. Composition of the window. Calculate Comodof the window. Co	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
5 Display the possible composition for feature #222.	s Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Compositions Export Chemistry Calculate Compositions Compositions Export Chemistry Calculate Compositions Compositions Compositions Calculate Compositions Compositions Compositions Compositions Compositions Calculate Compositions	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
5 Display the possible composition for feature #222.	s Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Compositions Export Chemistry Calculate Compositions Compositions Export Chemistry Calculate Compositions Compositions Compositions C37H61N7DE 0.0014 1.9 11.0 2 C23H53N230 0.0032 4.4 9.0 C31H65N501 0.0040 5.4 2.0 5 C39H55N15 0.0041 5.5 21.0 6 C41H65N70E 0.0047 6.5 6.0 8 C27H65N130 0.0054 7.4 2.0 9 C27H55N170 0.0059 8.1 8.0 10 C35H61N110 0.0061 8.3 11.0 11 C43H57N902 0.0067 9.2 20.0 11 C43H57N902 0.0067 9.2 20.0 12 20.0 12 20.0 12 22H57N170 0.0058 9.3 7.0 12 20.0 1	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide.
3 Display the possible composition for feature #222.	s Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Compositions Export Chemistry Calculate Compositions Compositions Export Chemistry Calculate Compositions Compositions Compositions C37H61N7DE 0.0014 1.9 11.0 2 C23H53N230 0.0032 4.4 9.0 C31H65N501 0.0040 5.4 2.0 5 C39H53N15 0.0041 5.5 21.0 6 C41H65N70E 0.0047 6.5 6.0 8 C27H65N130 0.0054 7.4 2.0 9 C27H57N170 0.0059 8.1 8.0 10 C35H61N110 0.0067 9.2 2.00 12 C28H61N170 0.0068 9.3 7.0 13 C28H5N1210 0.0072 9.9 13.0	 If you need to change the element selected to calculate the composition, see the Mass Hunter Quick Start Guide. ositions 97 42 78 71 70 82 65 65 68 47 43 80
3 Display the possible composition for feature #222.	s Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Compositions Export Chemistry Calculate Compositions Compositions Export Chemistry Calculate Compositions Compositions Compositions C37H61N7DE 0.0014 1.9 11.0 2 C23H53N230 0.0032 4.4 9.0 C31H65N501 0.0040 5.4 2.0 5 C39H53N15 0.0041 5.5 21.0 6 C41H65N70E 0.0047 6.5 6.0 8 C27H57N170 0.0054 7.4 2.0 9 C27H57N170 0.0057 7.4 2.0 10 C35H61N110 0.0067 8.3 11.0 11 C43H57N902 0.0067 9.2 20.0 12 C28H51N170 0.0068 9.3 7.0 13 C28H53N210 0.0072 9.9 13.0 14 C30H65N701 0.0073 9.9 2.0	 If you need to change the element selected to calculate the composition, see the Mass Hunte Quick Start Guide. ositions 97 42 70 82 63 42 65 68 47 43 80 68
<i>s</i> Display the possible composition for feature #222.	s Click Calculate. The possible compositions for the feature appear in a table at the bot of the window. Export Chemistry Calculate Compositions Export Chemistry Calculate Compositions Compositions Export Chemistry Calculate Compositions Compositions Calculate Compositions Compositions Compositions Compositions Compositions Calculate Compositions Compositions Compositions Compositions Compositions	 If you need to change the element selected to calculate the composition, see the Mass Hunte Quick Start Guide. ositions 97 42 70 82 63 42 65 68 47 43 80 68 57

Exercise 6 – Export Mass Profiler Feature Summary for GeneSpring

In addition to exporting almost all the tables, graphics and text information from Mass Profiler to an Excel file or .txt file for future use, you can also export the Feature Summary information in the Feature Table to a .txt file and upload the file to GeneSpring. You may want to do this to take advantage of GeneSpring's advanced filtering, normalization and differential analysis techniques or the capacity to compare more than two groups of features. You will, however, have to align the MS-produced features first with Mass Profiler before uploading the .txt file into GeneSpring.

This exercise shows you how to export the Feature Summary to a .txt file and how to use the information in GeneSpring.

St	eps	Detailed Instructions Comments	
1	Export the Feature Summary Table to a .txt file. • Call it "Myoglobin-Spikein.txt".	 a Select File > Export for GeneSpring. b Enter Myoglobin-Spikein, and click Save. 	
2	 Call It Myoglobin-Spikein.txt . In GeneSpring create a custom genome from Mass Profiler output file. Make sure headers for annotation file say Systematic Name, RT and Mass. Save the new genome as "Myoglobin Spike-in experiment". 	and Click Save.	
		Custom Settings There are one or more GenBank or EMBL files for my genome There is a single tab-delimited file containing all of my genes and annotation: Next Cancel Help	5

Steps D	etailed Instructions	Comments
C	Make sure that Create a Custom Genome is selected.	
d	Make sure that There is a single	
	tab-delimited file containing all of my	
	genes and annotations is selected.	
e	Click Next.	
f	Select the Mass Profiler output file,	
	Myoglobin-Spikein.txt, and click	
	Open.	
	The Import Genome: Annotations File	
	dialog box appears.	
g	In the Line of column titles text field,	
	use the up arrow to select 3, or simply	
	type in the number 3. Column Titles are	
	indicated with red bold type.	
h	Click on the Click to Set column	
	header and change this to Systematic	
	Name.	
i	Right-click the second column header,	
	and select RT from the list.	
j	Right-click the third column header,	
	and select mass from the list.	

Kimport Genome: / Please i column, does no pull-dow in the di	Annotations File choose the annotation type and the entries in that colu t contain annotations choo m menu choose "Custom" alog box that appears.	for each column. You Imn must be unique. If se "Ignore". If the anno and type the name of	must choose a "System: f a column in the annotat station type is not listed in the annotation (e.g. "Des	atic Name" ions file 1 the cription")	
Annotation Type	Systematic Name	RT	mass	Click to Set	
Line 1 (ignored)	Output from Agilent M				
Line 2 (ignored)	7688, 1, 10 = featureC	[
Line 3 (Column Titles)	ID	RT	mass	Nov29-61_1_1_1	
Line 4 (gene)	1	1392.789	1162.6243	2426.936	
Line 5 (gene)	2	961.177	1533.7464	2330.992	
Line 6 (gene)	3	1437.407	1880.8998	2443.646	
Line 7 (gene)	4	1289.094	1638.9329	2330.76	
Line 8 (gene)	5	1501.838	1419.6731	2031.394	
Line 9 (gene)	6	1175.219	1304.7103	2055.925	
Line 10 (gene)	7	1770.896	3512.6329	23.42523	
Line 11 (gene)	8	2555.471	414.2043	0.001	
Line 12 (gene)	9	2964.335	312.1367	1812.562	
Line 13 (gene)	10	1605.865	1478.7871	456.4213	-
Column Titles	column titles as annotation	inames Line of co	olumn titles 3	Res	et

- k Click Next.
- I When the warning appears, click Yes to continue.
- **m** Ignore the next window, and click Next.
- n Add any links you want (optional), and click Next.
- o In the Save New Genome window, enter the Name as Myoglobin Spike-in experiment.
- p Enter the Folder and Notes that you want.

Steps



Detailed Instructions Steps Comments 3 Populate the Myogobin Spike-in a Click the Myoglobin-Spikein.txt file experiment genome with Mass and drag it to the main GeneSpring Profiler data and define the data window containing the blank genome. format. b For Choose File Format, click the down Select a custom data format. • arrow and select **Custom** from the list. Assign the first column to be c Select Myoglobin Spike-in the Gene Identifier column. experiment from the genome Assign the sample (.mhd file) directory. abundance columns as Signal columns. 🌊 Import Data: Define File Format and Genome File Format Choose File Format: Custom -Genome Select the genome (set of genes on the array) for this data. If your genome does not appear on the list, you can create a new one by selecting Create a New Genome. Select Genome 🕂 🖾 Illumina 🖳 🔍 Whole Human Genome - IL 🔄 Proteomics –💫 CSF 25 Samples 🐁 Myoglobin spike in 🗞 Myoglobin Spike-in experimer –🐁 Pierre Thibault 6 spike-in Ġ Rat – 💫 Custom Rat Hamadeh -S. Rat NIEHS v1 C Create a New Genome Help Next.. Cancel

d Click Next.

The Import Data: Column Editor appears.

- e Click the Click to Set column header for the ID column, and select Gene Identifier.
- f Click the **Click to Set** column header for the first abundance column (.mhd file name), and select **Signal**.
- g Repeat step f for the second sample.

eps		Deta	iled Instructio	ns	Con	nments		
		h C a: fi	lick Guess The s the header fo le abundance c	Rest to assign r the rest of the olumns.	n Signal ne .mhd			
🕅 Import Data: Coli	umn Editor							
[
Step 1: Assign functions f	to columns in	your data file. You r	nust assign a "Gen	e Identifier" columi	n and at least one "S	Signal" column.		
Step 2: If your data file ha	is a row of col	umn titles directly a	bove the expressior	i data, select this n	ow using the control	s in the "Colum	n Titles" panel.	
Step 3: If your file has a "F	Flags" columr	, enter the values th	nat will appear in tha	at column into the "	Flag Values" panel I	below.		
Step 4: If you might be loa	ading files of t	his format in the fut	ure, click "Rememb	er this Format". Th	is option is not avail	able for formats	with multiple sign	al columns
Differences	Course & Donald	Test Control in a sec	- Transferration	(Henry)	Cimal	(Though	Rind	
Line 1 (ignored)	Output from A	cilent Molecular Pr	ofiler at 10/26/2005	10-47-20 AM	signal _	2010101	STUTIO	
Line 1 (ignored)	7688 1 10=	featureCount arou	nCount grounSize1	arounSize?				
Line 3 (Column Titles)	ID	RT	mass	Nm/29-61 1 1	1 Nov29-62 1 1	1 Nov29-63 1	1 1 Nov29-64 1	1 1 Nm/29
Line 4 (data)	1	1392,789	1162.6243	2426.936	2495.243	2367,808	2336.878	2324.3
Line 5 (data)	2	961.177	1533.7464	2330.992	2298.082	2296.129	2288.736	1890.9
Line 6 (data)	3	1437.407	1880.8998	2443.646	4.265583	2523.224	2499.315	2476.8
Line 7 (data)	4	1289.094	1638.9329	2330.76	1.33367	2259.55	2247.896	2221.9
Line 8 (data)	5	1501.838	1419.6731	2031.394	2149.152	2198.951	2232.816	0.6576
Line 9 (data)	6	1175.219	1304.7103	2055.925	2056.343	1987.647	1973.302	1944.9
Line 10 (data)	7	1770.896	3512.6329	23.42523	0.001	6.319585	0.001	70.977
More	more data is	included in the file t	han is shown here					
۹								
Function Gu Guess Th Clear G	uessing ne Rest uess	Column Titles Has Column Titl Line of Column T	es Titles 3	Pre Abs Mar	y Values sent Flag P sent Flag A rginal Flag M	Clear Rememb Advand	All Settings er This Format ced Options	
								-,

window appears.

The Import Data: Sample Attributes

a Click New Attribute.

Comments

4 Annotate the samples with their concentrations.

Steps

- Add an attribute (column) called Myoglobin concentration.
- Delete all other columns except for Sample Name.

New Attribute
New Attribute
Custom Attribute
C Choose Attributes from Standard List
Age Array Design Author Common Reference (Yes/No, ID) Concentration Data processing/normalization Developmental Stage Diseased/Normal Drug/Small-molecule Dye Swap Experiment Type Genetic Characteristic Growth Conditions Image Analysis Software Individual Identifier Labeling Protocol Organ/Organism part Organism Organization RNA type Sample Source Sampling Method Sex Strain/Cell-line Temperature Time Tissue Type Treatment type
OK Cancel

b Select **Custom Attribute**, if necessary, and click **OK**.

A new empty column is added.

- c Fill in the new column with Myoglobin Concentration and individual concentrations for each sample in fmol.
- **d** Highlight each of the other columns in turn, and click **Delete Attribute** until all columns are deleted except Sample.

ps	Det	ailed Instructions		Comments	
Import I	Data: Sample Attributes				
		Please select values fo	r sample attributes.		
	Sample Name				New Attribute
Attribute Nar		Myoglobin concentration			
Attribute Unit		fmol			Edit Attribute Valu
Numeric		yes			Delete Attribute
1	Myoglobin-Spikein.txt Nov29-61_1_1_1	30			
2	Myoglobin-Spikein.txt Nov29-62_1_1_1	30			Replace Text
3	Myoglobin-Spikein.txt Nov29-63_1_1_1	30			
4	Myoglobin-Spikein.txt Nov29-64_1_1_1	30			Fill Down
5	Myoglobin-Spikein.txt Nov29-65_1_1_1	30			Fill Sequence Do
6	Myoglobin-Spikein.txt Nov29-71_1_1_1	120			Cast
7	Myoglobin-Spikein.txt Nov29-72_1_1_1	120			
8	Myoglobin-Spikein.txt Nov29-73_1_1_1	120			
9	Myoglobin-Spikein.txt Nov29-74_1_1_1	120			
10	Myoglobin-Spikein.txt Nov29-75_1_1_1	120			
		Previous Next	Cancel Help		

The Import Data: Create Experiment

message appears.

Steps	Detailed Instructions	Comments	
 Create a new experiment called "Myoglobin Spike-in experiment". Add a new myoglobin concentration parameter to the experiment parameters. Change the interpretation settings and save the interpretation to a file names 30 	 a Click Yes to create with the ten sampl annotated. The Save New Exp appears. b Type in the Name of Myoglobin Spike-it 	a new experiment es you just eriment dialog box of the experiment, i n experiment .	
vs 120:	Save New Experime	ent	
– Sample: Do Not Display	Name Myoglobin Spike-in	experiment	
 Myoglobin Concentration: 	Folder		
Continuous	Project		Change Project(s)
	Notes		
	E- Experiments	Sample Name	<u>^</u>
		1 Myoglobin-Spikein.txt Nov29-61_1_1	1
		2 Myoglobin-Spikein.txt Nov29-62_1_1_	1
		3 Myoglobin-Spikein.txt Nov29-63_1_1	1
		4 Myoglobin-Spikein.txt Nov29-64_1_1	1
		6 Myoglobin-Spikein.txt Nov29-71 1 1	1
		7 Myoglobin-Spikein.txt Nov29-72_1_1_	1
		8 Myoglobin-Spikein.txt Nov29-73_1_1_	1
		9 Myoglobin-Spikein.txt Nov29-74_1_1_	_1
		10 Myoglobin-Spikein.txt Nov29-75_1_1_	_1
		1	*
		Save Cancel	

c Click Save.

The New Experiment Checklist dialog box appears.

🔍 New Experiment Checklist						
You are al should se choose yo the button:	most finished creating your experime t up its normalizations, experimental j ur default experiment interpretation. Y s below. Alternatively, you may find the	nt. Before you begin analysis, you parameters, and error model, and iou may reach these windows using em in the Experiments menu.				
New Experimen	t Checklist					
	Define Normalizations	Normalizations				
	Define Parameters	Parameters				
	Define the Default Interpretation	Experiment Interpretation				
	Define the Error Model	Error Model				
	Close					

- **d** To add parameters, click **Parameters**. The Experiment Parameters dialog box appears.
- e Click Import Parameter....

🎗 Import Parameters		×
Import Parameters from Sample Attrib	utes	
		Select All Clear All
Import Parameters from other Experim	ents	
Experiments	Parameters from Selected Experiment:	
└ <mark>:</mark> II Myoglobin Spike-in experi	Please select an experiment from the navigator.	Select All Clear All
	OK Cancel Help	

f Make sure that Myoglobin concentration is selected, and click OK.

Steps

g Select the File Name column, and click Delete Parameter. The Experiment Parameters table is

now complete.

Column	Myoglobin	New Parameter
	free al	
	Imoi	Import Parameter
no	yes	Delete Revemeter
N/A	no	Delete Parameter
Nov29-61_1_1	_1 30	
Nov29-62_1_1_	_1 30	Replace Text
Nov29-63_1_1_	_1 30	Extract Subvalues
Nov29-64_1_1_	_1 30	Fill Down
Nov29-65_1_1_	_1 30	Fill Sequence Dow
Nov29-71_1_1	1 120	Sort
Nov29-72_1_1_	_1 120	
Nov29-73_1_1_	1 120	Ret Value Order
Nov29-74_1_1_	1 120	
Nov29-75_1_1_	1 120	inspect
	NJA Nov29-61_1_1 Nov29-61_1_1 Nov29-63_1_1 Nov29-64_1_1 Nov29-65_1_1 Nov29-65_1_1_1 Nov29-71_1_1 Nov29-73_1_1_1 Nov29-75_1_1 Nov29-75_1_1 Nov29-75_1_1 Nov29-75_1_1	N/A no Nov29-61_1_1_1 30 Nov29-62_1_1_1 30 Nov29-63_1_1_1 30 Nov29-64_1_1_1 30 Nov29-65_1_1_1 30 Nov29-65_1_1_1 30 Nov29-71_1_1_1 120 Nov29-73_1_1_1 120 Nov29-74_1_1_1 120 Nov29-75_1_1_1 120

h Click Save.

The New Experiment Checklist appears again. The **Define Parameters** check box is automatically marked, and the new settings are saved.

i Click Experiment Interpretation. The Default interpretation for the

Change Interpretation dialog box appears.

- j Change the Name to 30 vs 120.
- k Click Save As... Enter the name 30 vs 120. Click OK.
- I For the Display Parameters, select **Do Not Display** for Sample and **Continuous** for Myoglobin concentration.

Comments



- m In the New Experiment Checklist, click Close.
- n In the GeneSpring main navigator, select Experiments > Myoglobin
 Spike-in experiment > 30 vs 120 to see the result of changing the interpretation.



Steps

Other GeneSpring analyses available for Mass Profiler data:

- Scatter plot
- Volcano plot
- Fold change filter and plot
- ANOVA
- Venn Diagrams
- Clustering
- Hieriarchical Trees
- Principle Least Squares Discriminant Analysis (PLS-DA)
- Principle Component Analysis (PCA) See plot below.



www.agilent.com

In this guide

This Quick Start Guide includes an overview of the Mass Profiler software, quick reference information to get started using the software, and a set of tutorials to learn how to use the software.

© Agilent Technologies, Inc. 2005

Printed in USA First edition, November 2005



G3297-90001

