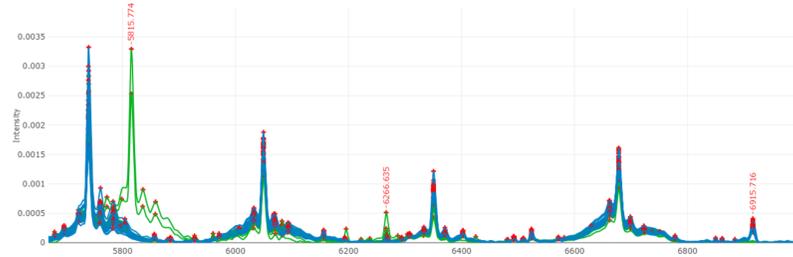


How to perform Biomarker Analysis in our platform?

CLOVER MS Data Analysis Software

What is it?



In Clover Biosoft, the **Biomarker Analysis** module allows you to search for **potential peaks** that can be used to identify or discriminate groups of samples. Indeed, from this analysis, the user can create a new peak matrix based on the peaks of interest for training machine learning algorithms.

1 First steps

This guide is only for MALDI spectra

1 Go to the **Bio Analysis** section and click on **Biomarker Analysis**

2 Choose a **Study**

3 Choose an **Experiment**

2 Biomarker Analysis steps

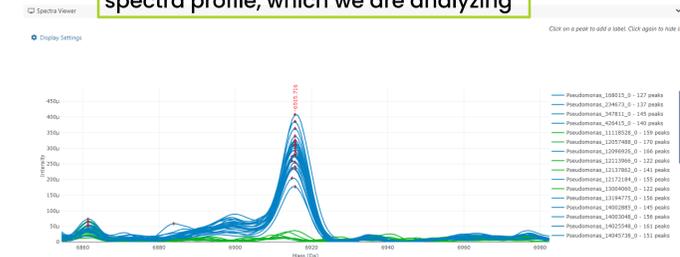
1. Choose the **categories** that you want to analyse

2. Apply the alignment and the tolerance with **default** or **custom** parameters, and find peaks

3. Analyse the **results**: Spectra Viewer, Peak Distribution Table, Univariate Analysis, Peak Roc, and so on

3 Biomarker Analysis results

Spectra Viewer: a visualizer for the spectra profile, which we are analyzing



Peak Distribution Table: peak list with its percentage of appearance and metrics per category

Mass (z)	Number of spectra (n)	Appearance %	CV %	Outbreak	Mean (z)	STDEV (z)	Appearance %	CV %	Control	Mean (z)	STDEV (z)
20015460	13	1528 (37.8%)	+ 35.561%	2.899e-4	5.975e-5	0.8 (0.0%)	-	-	0.000e+0	0.000e+0	0.000e+0
20014022	4	228 (27.0%)	+ 14.421%	7.879e-5	1.274e-5	2.8 (20.0%)	+ 11.303%	6.029e-5	5.149e-6	0.000e+0	0.000e+0
20123860	9	728 (20.0%)	+ 12.341%	8.770e-5	1.286e-5	2.8 (20.0%)	+ 10.44%	3.820e-5	3.720e-6	0.000e+0	0.000e+0
20181314	24	2428 (30.0%)	+ 32.273%	1.039e-4	1.580e-5	0.8 (0.0%)	+ 0.0%	5.150e-5	0	0.000e+0	0.000e+0
20224816	1	0.00 (0.0%)	-	-	-	1.8 (13.3%)	+ 0.0%	5.150e-5	0	0.000e+0	0.000e+0
20242796	6	628 (10.0%)	+ 33.300%	8.510e-5	2.837e-5	0.8 (0.0%)	+ 0.0%	5.150e-5	0	0.000e+0	0.000e+0
20348859	12	1128 (10.0%)	+ 15.380%	5.721e-5	9.074e-6	1.8 (13.3%)	+ 0.0%	4.420e-5	0	0.000e+0	0.000e+0
20413873	23	1728 (30.0%)	+ 12.280%	6.424e-5	9.828e-6	6.8 (29.1%)	+ 44.483%	1.162e-4	8.089e-5	0.000e+0	0.000e+0
20413764	3	128 (10.0%)	+ 0.0%	9.327e-5	1.0	2.8 (20.0%)	+ 44.44%	1.274e-4	1.670e-5	0.000e+0	0.000e+0
20512512	28	2528 (30.0%)	+ 11.287%	1.039e-4	1.378e-5	4.8 (16.7%)	+ 11.366%	8.491e-5	1.039e-5	0.000e+0	0.000e+0

Univariate Analysis: t-Test or Mann-Whitney U test for two categories; one-way ANOVA for three or more categories

Mass (z)	p-value (z)	q-value (FDR adjusted p-value) (z)
69153669	1.220e-20	4.079e-18
20054251	5.100e-14	1.080e-11
110343484	9.150e-13	1.020e-10
21078905	3.079e-12	3.100e-10
20161314	2.850e-11	2.110e-9
34573235	4.470e-11	3.000e-9
102468115	6.880e-11	3.850e-9
20563376	1.830e-10	5.960e-9
38053792	1.210e-10	5.960e-9
2044315	2.870e-10	9.600e-9

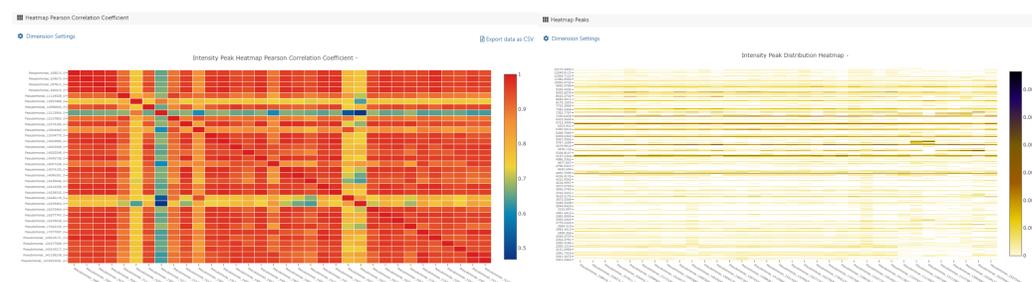
4 **Optional: Generation of the peak matrix**

Selected Biomarkers
2081.7323 2556.3376 6915.5669 8441.6646
Save Peak Matrix

Group pair comparison: by appearance or intensity mean

Mass (z)	Outbreak - Control (z)
20015460	5.138
20014022	15.31
20123860	1.82
20181314	92.31
20224816	12.50
20242796	23.08
20348859	23.81
20413873	9.82
20413764	21.15
20512512	46.19

Heatmaps: Pearson Correlation Coefficient and Intensity Peak Distribution



Peak ROC

Peak (z)	AUC (z - 0.5)	Appearance (z)	Positive Category (z)
69153669	0.9952	26/34	26/26
2081.7323	0.9952	29/34	26/26
8441.6646	0.9904	30/34	26/26
2556.3376	0.9904	29/34	26/26
3457.5235	0.9904	27/34	26/26
2391.0108	0.9856	29/34	26/26
6370.9057	0.9856	32/34	26/26
13249.8115	0.9856	27/34	26/26
2107.8905	0.9794	26/34	25/26
2436.8347	0.9794	26/34	25/26



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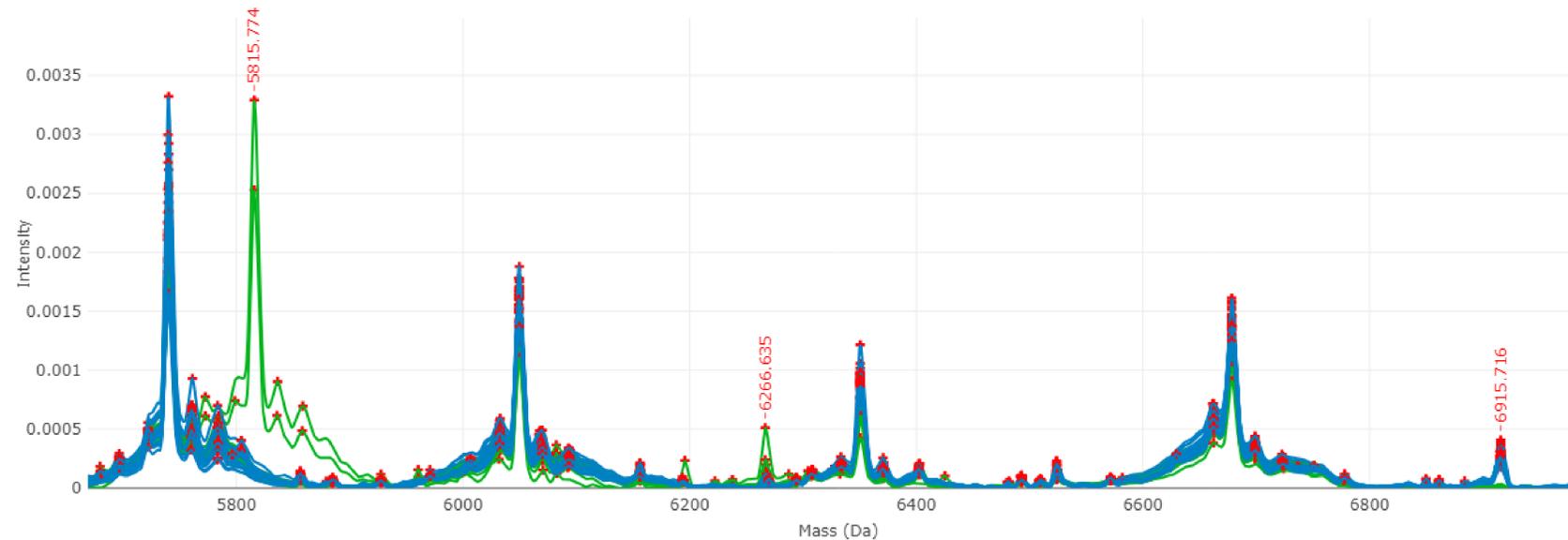
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Clover MS Data Analysis Software, Quick Start Guide



What is it?



In Clover Biosoft, the **Biomarker Analysis** module allows you to search for **potential peaks** that can be used to identify or discriminate groups of samples. Indeed, from this analysis, the user can create a new peak matrix based on the peaks of interest for training machine learning algorithms.



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Clover MS Data Analysis Software, Quick Start Guide

How to perform Biomarker Analysis in our platform?

CLOVER MS Data Analysis Software

1

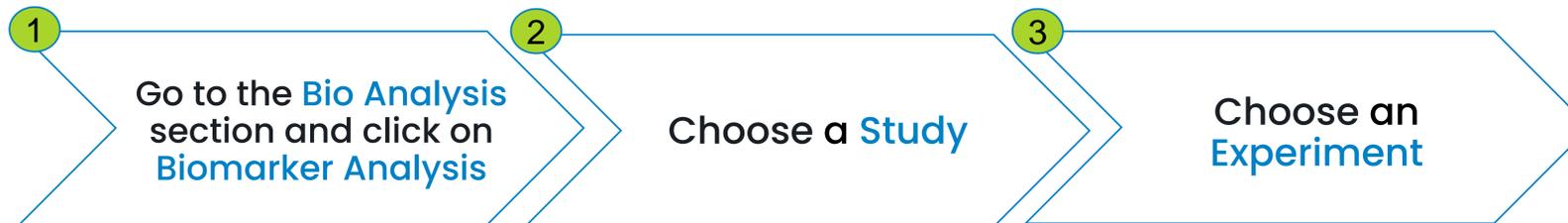
First steps



This guide is only for MALDI spectra

The screenshots illustrate the navigation process:

- Step 1:** The main dashboard of the software. The 'Bio Analysis' menu item in the left sidebar is highlighted with a green circle and the number '1'. An arrow points from this circle to the 'Biomarker Analysis' card on the dashboard.
- Step 2:** The 'Biomarker Analysis - Select Study' screen. A green circle with the number '2' is positioned over the 'Select Study' button.
- Step 3:** The 'Biomarker Analysis - Select Experiment' screen. A green circle with the number '3' is positioned over the 'Select Experiment' button.



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Clover MS Data Analysis Software, Quick Start Guide

2

Biomarker Analysis steps

1. Choose the **categories** that you want to analyse

2. Apply the alignment and the tolerance with **default** or **custom** parameters, and find peaks

3. Analyse the **results**: Spectra Viewer, Peak Distribution Table, Univariate Analysis, Peak Roc, and so on

The screenshots illustrate the following steps:

- Step 1:** The 'Categories' section shows two categories: 'Outbreak' and 'Control'. A list of 26 samples is displayed below, including Pseudomonas strains like Pseudomonas_168015_0 and Pseudomonas_234673_0.
- Step 2:** The 'Find Peaks' section shows configuration options for alignment (By threshold, By mass position, By number of peaks) and tolerance (0,02). A 'Do Find Peaks' button is visible.
- Step 3:** The 'Result' section displays a 'Peak Distribution Table' comparing 'Outbreak' and 'Control' groups. The table includes columns for mass, number of spectra, appearance, CV, mean, and STDEV.

Mass + 1	Number of spectra	Outbreak				Control			
		Appearance	CV	Mean	STDEV	Appearance	CV	Mean	STDEV
2001.5466	15	15/26 (57.69%)	+	35.581 %	2.689e-4	9.567e-5	0/8 (0.0%)	-	-
3.4082	4	2/26 (7.69%)	+	16.452 %	7.605e-5	1.251e-5	2/8 (25.0%)	+	13.385 %
7.23888	9	7/26 (26.92%)	+	15.341 %	8.370e-5	1.284e-5	2/8 (25.0%)	+	10.46 %
2018.1314	24	24/26 (92.31%)	+	32.273 %	1.109e-4	3.580e-5	0/8 (0.0%)	-	-
2020.4816	1	0/26 (0.0%)	-	-	-	-	1/8 (12.5%)	+	0.0 %
2024.2796	6	6/26 (23.08%)	+	33.308 %	8.512e-5	2.835e-5	0/8 (0.0%)	-	-



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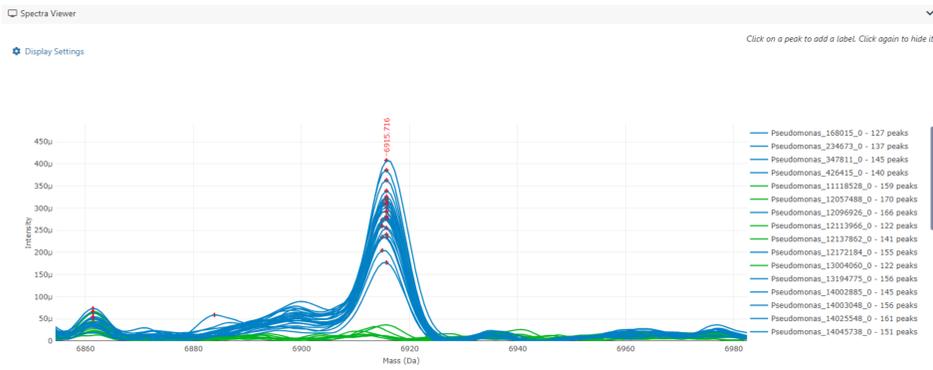
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3 Biomarker Analysis results

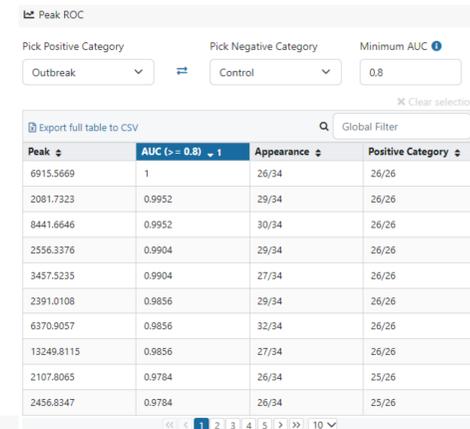
Spectra Viewer: a visualizer for the spectra profile, which we are analyzing



Peak Distribution Table: peak list with its percentage of appearance and metrics per category

Mass	Number of spectra	Outbreak				Control			
		Appearance	CV	Mean	STDEV	Appearance	CV	Mean	STDEV
2001.5466	15	15/26 (57.69%)	+	35.581 %	2.689e-4	9.567e-5	-	-	-
2003.4082	4	2/26 (7.69%)	+	16.452 %	7.605e-5	1.251e-5	2/8 (25.0%)	+	13.385 %
2012.3888	9	7/26 (26.92%)	+	15.341 %	8.370e-5	1.284e-5	2/8 (25.0%)	+	10.46 %
2018.1314	24	24/26 (92.31%)	+	32.273 %	1.109e-4	3.580e-5	0/8 (0.0%)	-	-
2020.4816	1	0/26 (0.0%)	-	-	-	-	1/8 (12.5%)	+	0.0 %
2024.2796	6	6/26 (23.08%)	+	33.308 %	8.512e-5	2.835e-5	0/8 (0.0%)	-	-
2034.8659	12	11/26 (42.31%)	+	15.86 %	5.721e-5	9.074e-6	1/8 (12.5%)	+	0.0 %
2041.3673	23	17/26 (65.38%)	+	15.299 %	6.424e-5	9.828e-6	6/8 (75.0%)	+	69.635 %
2043.5766	3	1/26 (3.85%)	+	0.0 %	9.327e-5	0	2/8 (25.0%)	+	44.449 %
2051.3512	29	25/26 (96.15%)	+	13.267 %	1.029e-4	1.379e-5	4/8 (50.0%)	+	15.366 %

Peak ROC



Univariate Analysis: t-Test or Mann-Whitney U test for two categories; one-way ANOVA for three or more categories

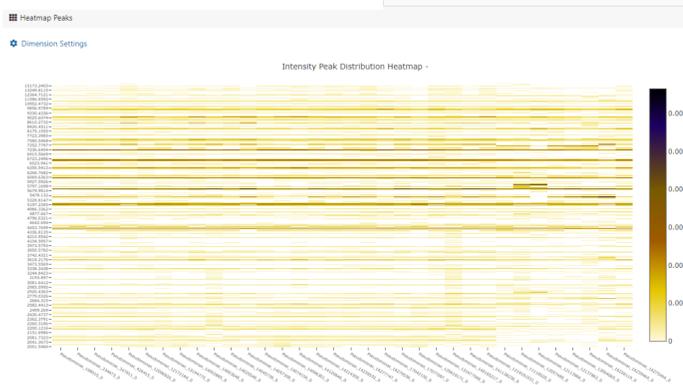
Mass	p-value	q-value (FDR adjusted p-value)
6915.5669	1.234e-20	4.972e-16
2090.4291	5.199e-14	1.048e-11
11934.3494	8.152e-13	1.095e-10
2107.8065	3.079e-12	3.102e-10
2018.1314	2.630e-11	2.119e-9
3457.5235	4.478e-11	3.008e-9
13249.8115	6.693e-11	3.853e-9
2556.3376	1.332e-10	5.966e-9
3850.5782	1.212e-10	5.966e-9
2684.315	2.397e-10	6.658e-9

Group pair comparison: by appearance or intensity mean

Mass	Outbreak - Control
2001.5466	57.69
2003.4082	17.31
2012.3888	1.92
2018.1314	92.31
2020.4816	12.50
2024.2796	23.08
2034.8659	29.81
2041.3673	9.62
2043.5766	21.15
2051.3512	46.15

Univariate Analysis: t-Test or Mann-Whitney U test for two categories; one-way ANOVA for three or more categories

Group pair comparison: by appearance or intensity mean



Heatmaps: Pearson Correlation Coefficient and Intensity Peak Distribution

4

Optional: Generation of the peak matrix

Selected Biomarkers

2081.7323 2556.3376 6915.5669 8441.6646

Save Peak Matrix

