

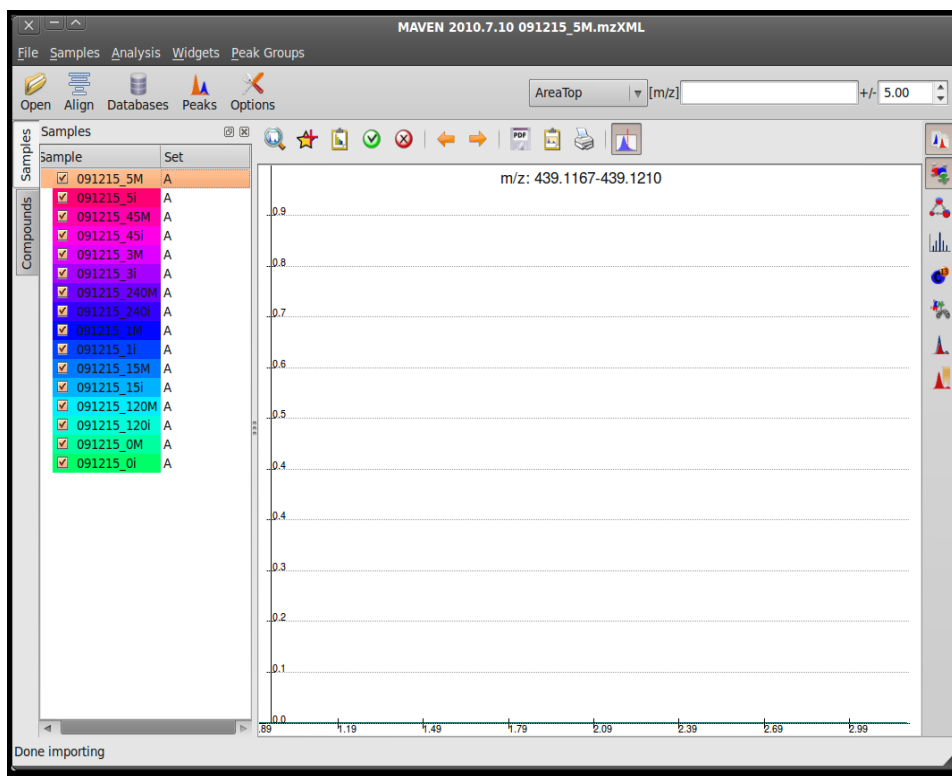
Getting Started.

This document provides basic overview of Maven functionality. For the purpose of demonstration we will use an example CMV Viral Infection time course dataset. ([Dataset Download](#)). There are sixteen mzXML files in this dataset, eight from viral infected samples and eight from mock infected samples. The samples were obtained using LC/MS setup with high resolution Thermo Exective instrument.

To get started download the dataset, unzip files, start Maven, and follow steps below.

Step 1: Load Samples and Create Project.

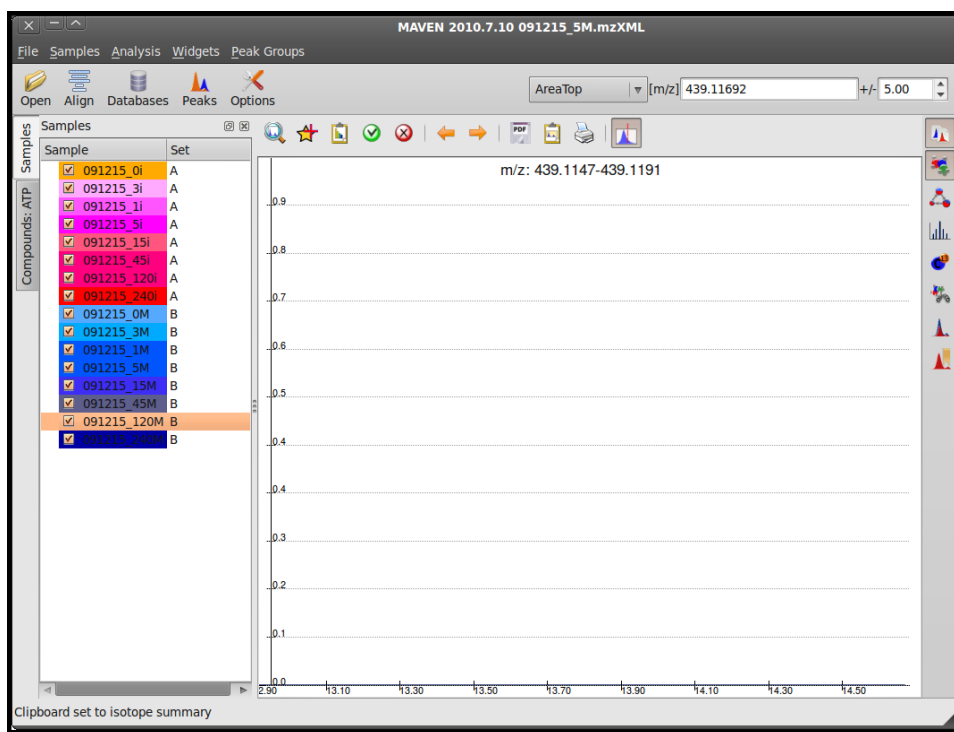
From file menu select “File” → “Load Samples” (“**Ctrl-O**”), and select all mzXML files from exampleProject folder. Once files have been loaded, click on “Show Samples Widgets” icon in the right sidebar, to see a color coded sample list with all the files that have been loaded.



To change a color of a sample double click on the name. To change sample

order drag samples up and down. Please note that all samples are currently assigned to the same group "A". To change the set name, double click on the set name in the second column. For example, lets change a set name for mock samples to set group "B". and lets change all viral samples to orange/red colors, and all mock samples to blue/purple colors.

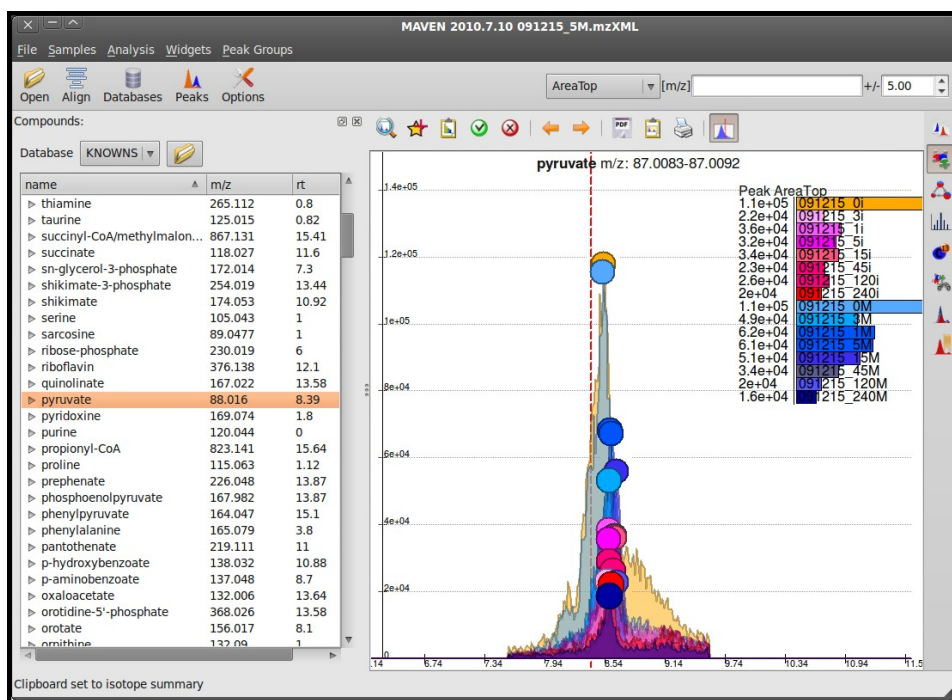
Now lets save the project ("File"->"Save Project" ("**Ctrl-S**") and examine metabolites.



Step 2: Examine Metabolites.



Click on "Compounds Widget" icon on the right sidebar to display the list of metabolites. The compound widget should become visible on the left side of the screen in the tab next to Samples Tab. The default list of metabolites displayed in the widget are from "KNOWNs" subset from "KNOWNs.csv" file. You can edit this file with any spreadsheet program if you wish to add metabolites, change retention times, or modify m/z values. Lets take a look at Extracted Ion Chromatogram (EIC) for Pyruvate.

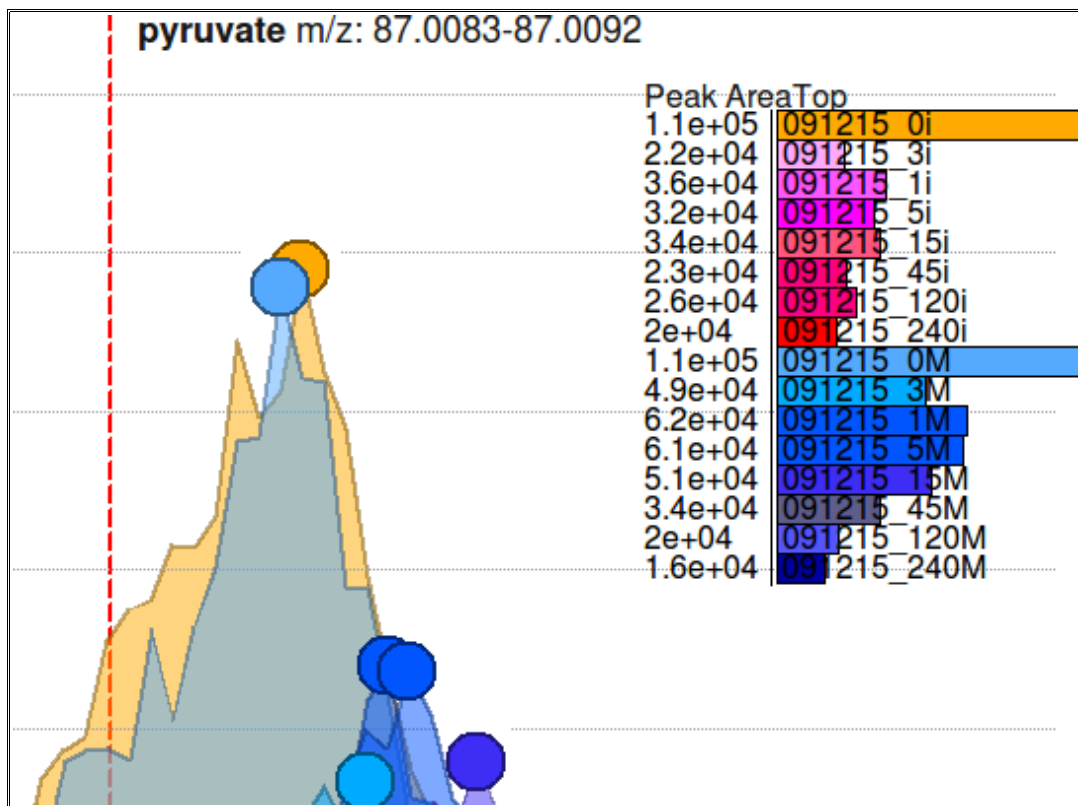


Since we are examining samples that were analyzed using negative ionization mode, the program will automatically calculate the expected m/z window (Mass of pyruvate - Hydrogen + electron) and create a chromatogram centered on a +/- 5 PPM window of the expected m/z. The PPM window can be adjusted in the upper right-hand corner of the screen.



By default, the program starts with "Auto Zoom" turned on. When Auto Zoom is enabled, the program will center the display on the expected retention time of the compound, in this case ~ 8.4 minutes.

Tip: To zoom in on the peaks, click and drag the cursor to the right, to unzoom click and drag to the left.



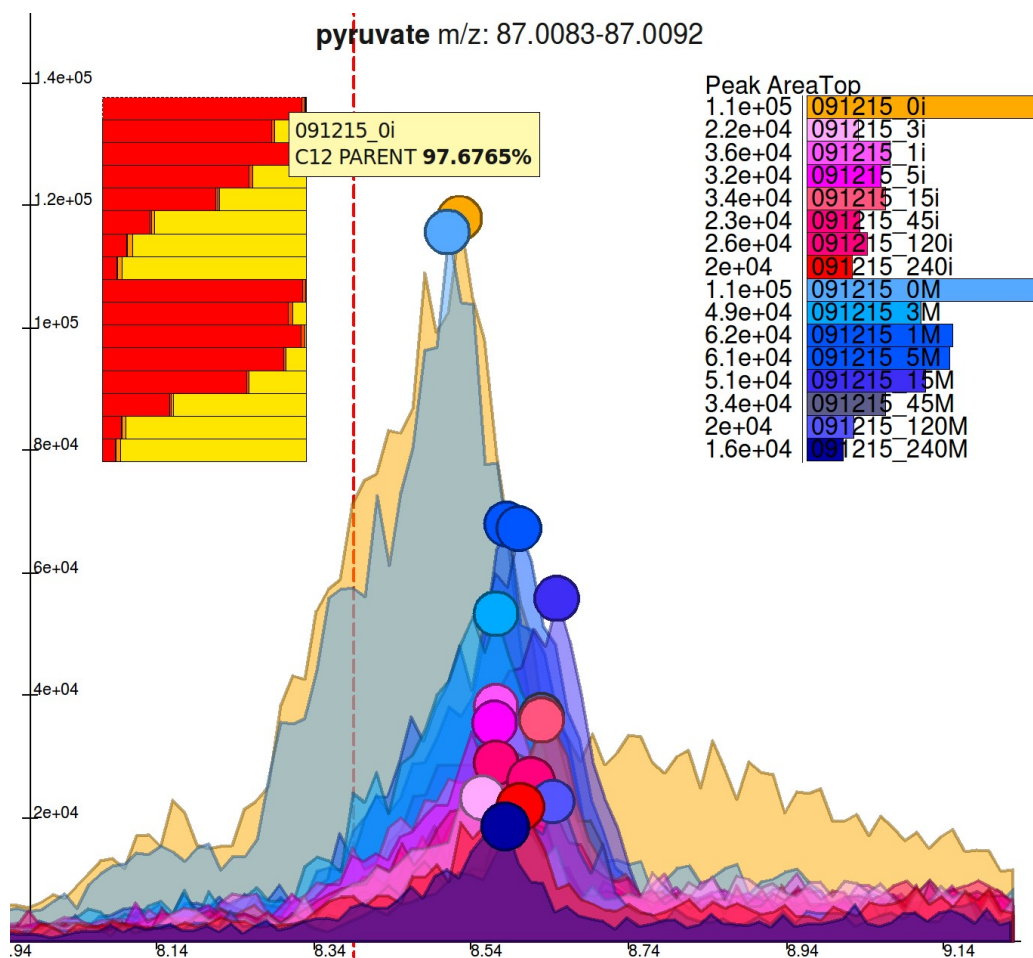
A zoom on a section of EIC plot is shown in the figure below. On the right side the program shows bar-chart with quantitative comparisons between samples. In this case, the measure we are using is “Peak AreaTop” - mean of three top points in the peak. Alternatively, you can switch to Peak Area or Peak Height measure by selecting an alternative from dropdown in the top toolbar.

The red dotted vertical line shows the expected retention time of the compound. The circle on top of each peaks shows the location of the peak and the quality of the peak. The quality is machine predicted measure of peak goodness ranging from 0.0 - poor quality , to 1.0 - good quality. The larger the circle the higher the quality. An analyst can train their own model to asses peak quality, more about that later.

Step 3. Extracting isotopic labeling data.

To extract isotopic labeling information, simply click once (left button single click) on any peak in the peak group. The program will calculate EICs for all

isotopic m/z in the region of the plot, calculate location of peaks, and quantitate peaks if there are any. It will then display bar chart on the left side of the screen with quantitative information.



The isotopic bar chart data will be automatically copied to clipboard. For example, after clicking on one of the peaks of the pyruvate, the clipboard will contain the following information.

| | A | B | C | D | E | F | |
|---|--------------------|-----------|-----------|-----------|-----------|------------|------|
| 1 | <u>pyruvate</u> | 091215_0i | 091215_3i | 091215_1i | 091215_5i | 091215_15i | 0912 |
| 2 | C12 PARENT | 108683.12 | 22472.16 | 35837.08 | 32051.62 | 33726.25 | 2 |
| 3 | C13-label-1 | 1925.98 | 351.18 | 503.39 | 711.23 | 639.13 | |
| 4 | C13-label-2 | 121.77 | 0 | 0 | 117.05 | 319.11 | |
| 5 | C13-label-3 | 537.53 | 4234.85 | 1412.27 | 11622.65 | 25838.45 | 7 |
| 6 | | | | | | | |
| 7 | | | | | | | |

The copy and paste into spreadsheet procedure works well if you wish to examine just few compounds. A faster method for storing information is to bookmark the peak group by left button double clicking on any peak in the group. All bookmarked groups will be save into Bookmarked Peak Groups Table, which can be exported to spreadsheet, saved for later used or loaded into another maven session. An example of Bookmarked Peak Group Table with three compounds and their isotopes is shown below.

| # | ▲ | Id | m/z | rt | #peaks | #good | Max Width | Max Intensity | Max S/N | Max Qu |
|-----|---|---------------|----------|-------|--------|-------|-----------|---------------|---------|--------|
| ▼ 0 | | pyruvate | 87.0087 | 8.59 | 16 | 16 | 132 | 1.2e+05 | 2615 | 0.84 |
| 0 | | C12 PARENT | 87.0087 | 8.59 | 16 | 16 | 91 | 1.2e+05 | 20 | 0.80 |
| 0 | | C13-label-1 | 88.0121 | 8.58 | 16 | 11 | 57 | 2.9e+03 | 292 | 0.83 |
| 0 | | C13-label-2 | 89.0155 | 8.59 | 10 | 6 | 55 | 7.7e+03 | 774 | 0.84 |
| 0 | | C13-label-3 | 90.0188 | 8.56 | 16 | 14 | 95 | 2.6e+05 | 94 | 0.82 |
| ▼ 0 | | riboflavin | 375.1314 | 12.68 | 16 | 14 | 13 | 1e+04 | 1025 | 0.85 |
| 0 | | C12 PARENT | 375.1313 | 12.68 | 16 | 15 | 13 | 1e+04 | 1025 | 0.85 |
| 0 | | C13-label-1 | 376.1346 | 12.66 | 14 | 5 | 10 | 2e+03 | 196 | 0.83 |
| 0 | | C13-label-2 | 377.1379 | 12.65 | 2 | 0 | 1 | 4.6e+02 | 46 | 0.07 |
| ▼ 0 | | phosphoeno... | 166.9752 | 15.25 | 13 | 5 | 22 | 4.6e+03 | 459 | 0.85 |
| 0 | | C12 PARENT | 166.9752 | 15.25 | 13 | 4 | 22 | 4.6e+03 | 459 | 0.85 |
| 0 | | C13-label-2 | 168.9819 | 15.32 | 1 | 0 | 2 | 2.5e+02 | 25 | 0.09 |
| 0 | | C13-label-3 | 169.9853 | 15.28 | 10 | 9 | 20 | 5.8e+03 | 585 | 0.84 |