



EVA

Installation and User Instructions

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In rare cases, EVA GUI might disappear after the prediction is completed due to R package version conflicts. In these cases, the prediction outcome is already generated and exists in the backend folder (C:/Users/User/AppData/Local/Packages/11888HuanLab.EVAanalysisistool_8tmgtde19meca/LocalState).

We provide an R code (EVAgeneratingResultsTable.r) to combine the prediction outcome with the feature table, and generate the final result for downstream data interpretation.



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1 Installation

1.1 Download

Download the EVA app from the Microsoft Store by searching “EVA Metabolomics” (Figure 1.1). Download the supplementary files including Python37, R-4.0.4, user manual, and demo data from the Huan Lab Google Drive (Figure 1.2) at:

https://drive.google.com/drive/folders/189v_Bn7KbTyrKWCZUq7Jc6f-g6Q1qVSS?usp=sharing.

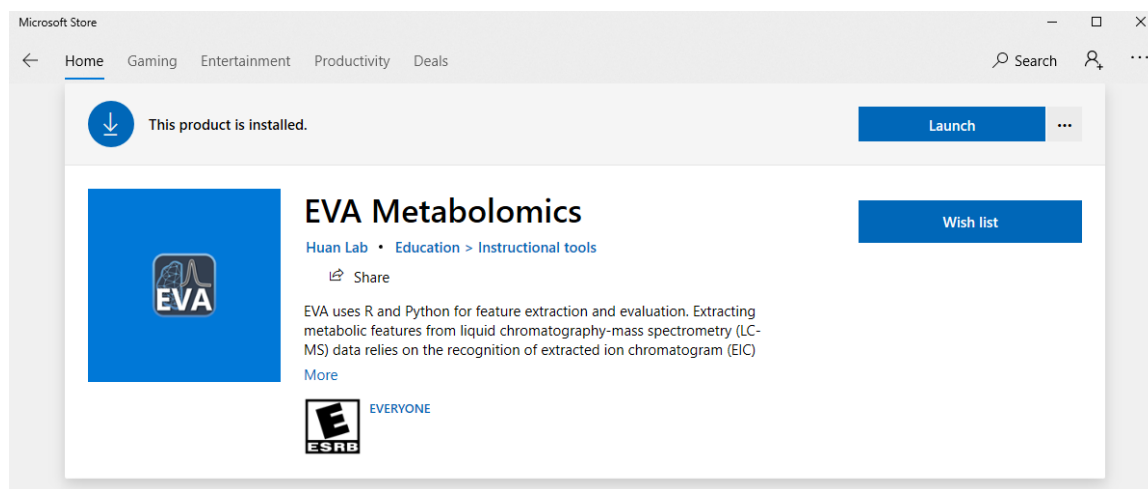


Figure 1.1: Microsoft Store listing for EVA.

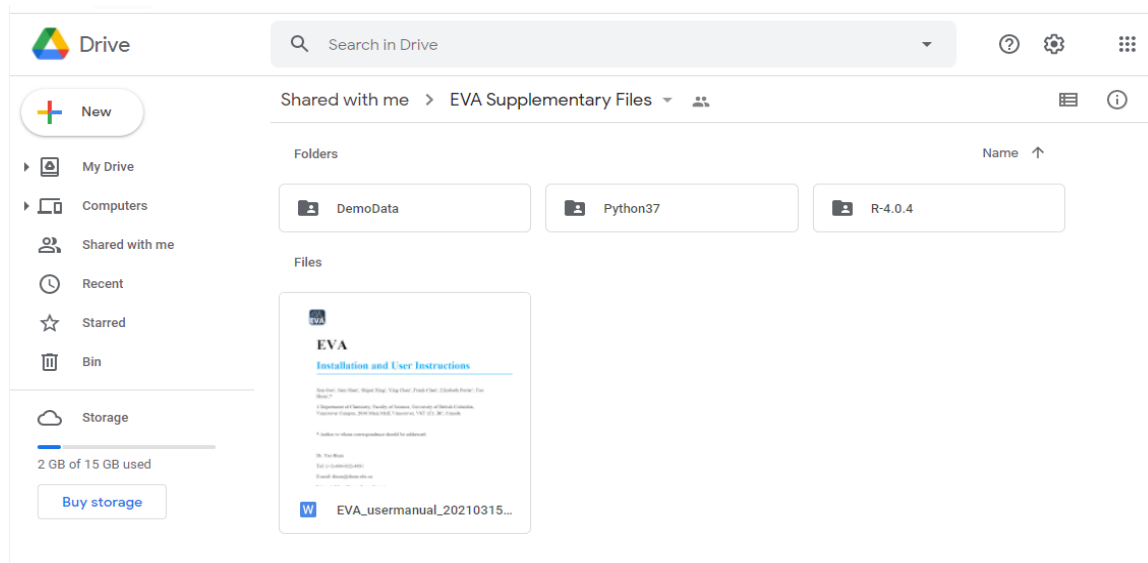


Figure 1.2: EVA supplementary files Google Drive.



2 Usage

2.1 Open EVA

Once installed, EVA can be found in the Windows menu or search bar as shown in Figure 2.1 below.

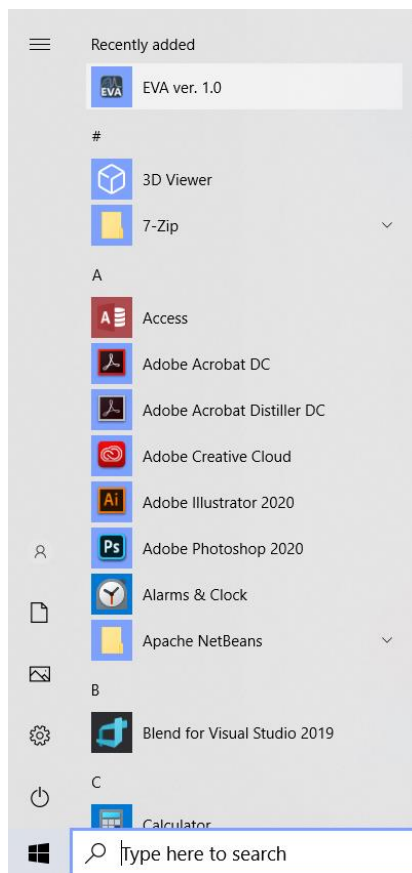


Figure 2.1: EVA app in the search bar.

2.2 Setup R Environment

EVA uses R to extract and plot chromatograms. R 3.5.2 or later with packages “XCMS”, “ggplot2”, “scales”, and “dplyr” need to be installed on your computer prior to using EVA. If you would like to use the R package provided in the supplementary files folder with preinstalled packages, then the rest of this step can be skipped.



If you choose to use your own R, we recommend upgrading your R version to 3.5.2 or later and updating the required R packages to the latest version using RStudio. If you do not have RStudio or R on your computer, you can download R at <https://cran.rstudio.com/> and RStudio at <https://rstudio.com/products/rstudio/download/#download>.

Next, install or update these required R packages by copy-pasting the following lines of code into either R Console or RStudio:

1. *if (!requireNamespace("BiocManager", quietly = TRUE))
install.packages("BiocManager")*
2. *BiocManager::install("xcms")*
3. *install.packages("ggplot2")*
4. *install.packages("scales")*
5. *install.packages("dplyr")*

During the installation process, follow the prompt to update or install all dependency packages as well. If error occurs during installation, follow the on-screen instructions in R Console or RStudio to troubleshoot. Once the packages are properly installed or updated, check if they can be properly loaded by copy-pasting the following lines of code into either R Console or RStudio:

1. *library(xcms)*
2. *library(ggplot2)*
3. *library(scales)*
4. *library(dplyr)*

If any error message(s) occurs, make sure to reinstall the package(s) that failed to load. Warning messages can be disregarded.

Note:

This step only needs to be done once if you don't have R or these R packages.

2.3 Setup Python & R Executable

EVA uses both python and R; users must set the path to their Python and R exe files prior to running EVA. To do so, follow the steps below:

1. Click on the “gear” shown in Figure 2.2 to enter the settings page (shown in Figure 2.3).

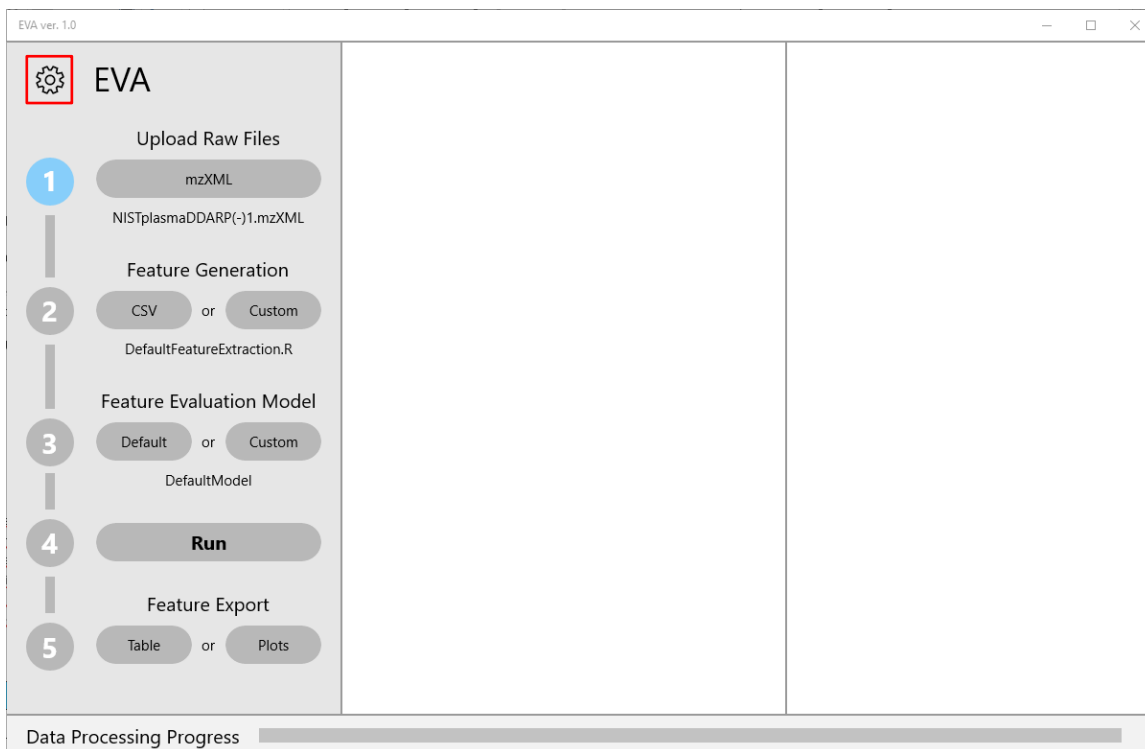


Figure 2.2: EVA interface.

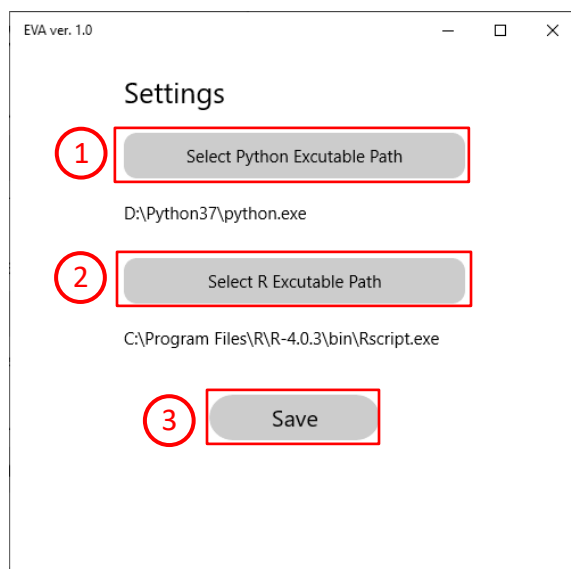


Figure 2.3: EVA Settings Page.



2. In “Settings” window, click on “Select Python Executable Path” and “Select R Executable Path” to browse and select the “python.exe” and “Rscript.exe” files respectively. An example is shown in Figure 2.4 and Figure 2.5.
 - a. The Python exe file is located in the Python37 folder downloaded from Google Drive:
`...\EVA_1.0_install\Python37\python.exe`
 - b. The Rscript exe file’s location varies depending on how you installed R. If you followed R’s standard installation process, the “Rscript.exe” file is usually located at:
`C:\Program Files\R\R-4.0.3(varies depending on your R version)\bin\Rscript.exe`
If you used the R package from Google Drive, you can find the “Rscript.exe” file at:
`...\EVA_1.0_install\R-4.0.4\bin\Rscript.exe`

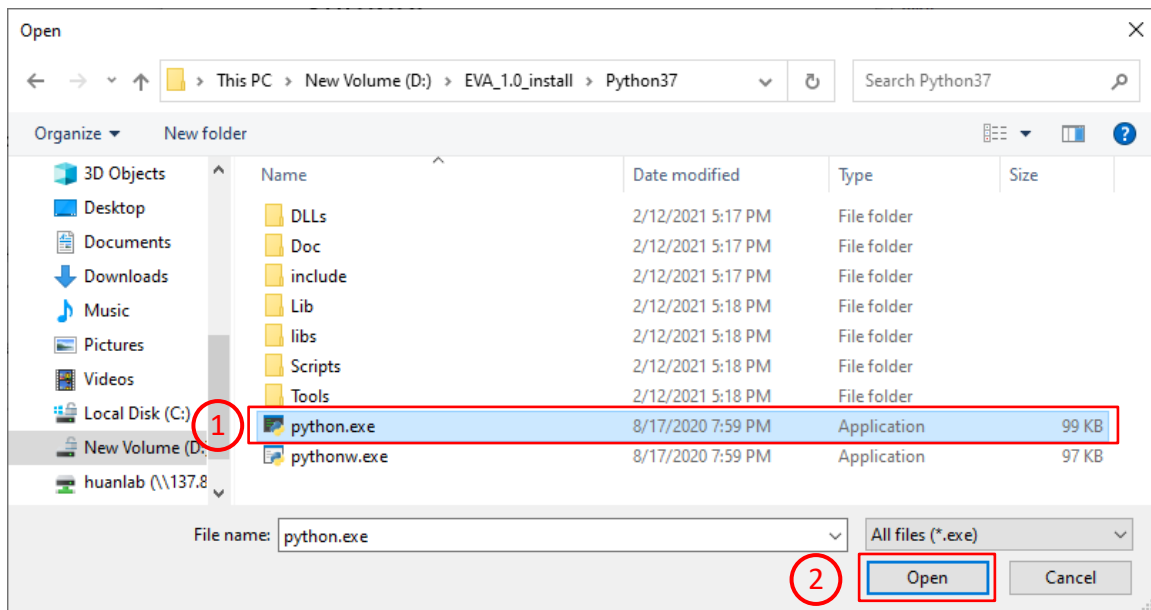


Figure 2.4: Interface to select Python exe file.

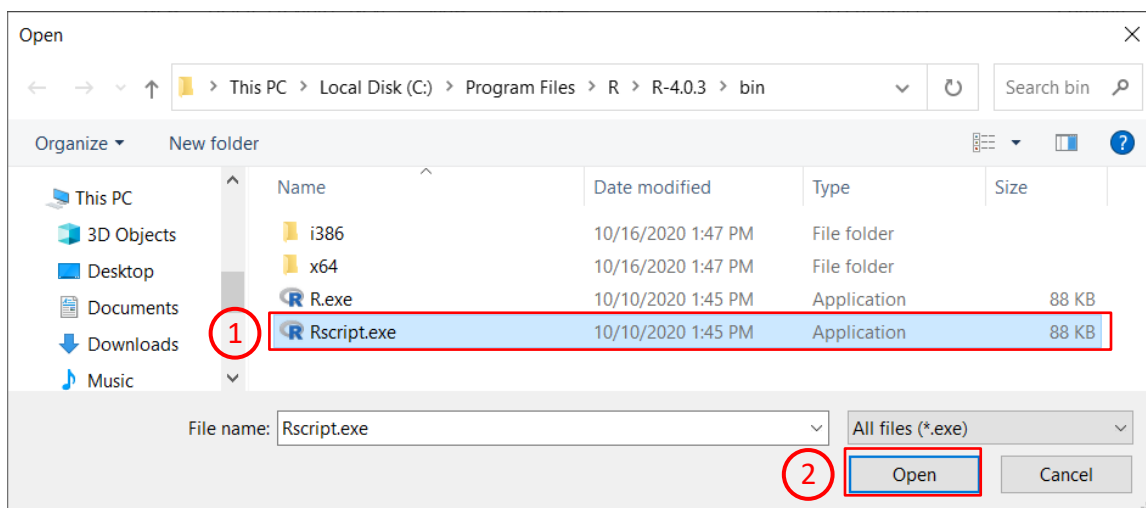


Figure 2.5: Interface to select Rscript exe file.

3. Once the paths of the 2 exe files are selected, click on “Save”, then close the “Settings” window.

Note:

This step only needs to be done once when the application is newly installed. If you changed the location of your Python or R exe files, redo this step.

2.4 Upload Files

2.4.1 Upload mzXML Files

Click on the “mzXML” button and select 1 or multiple mzXML files to upload as shown in Figure 2.6 and 2.7 respectively. Preview your selected files in the window below the button.

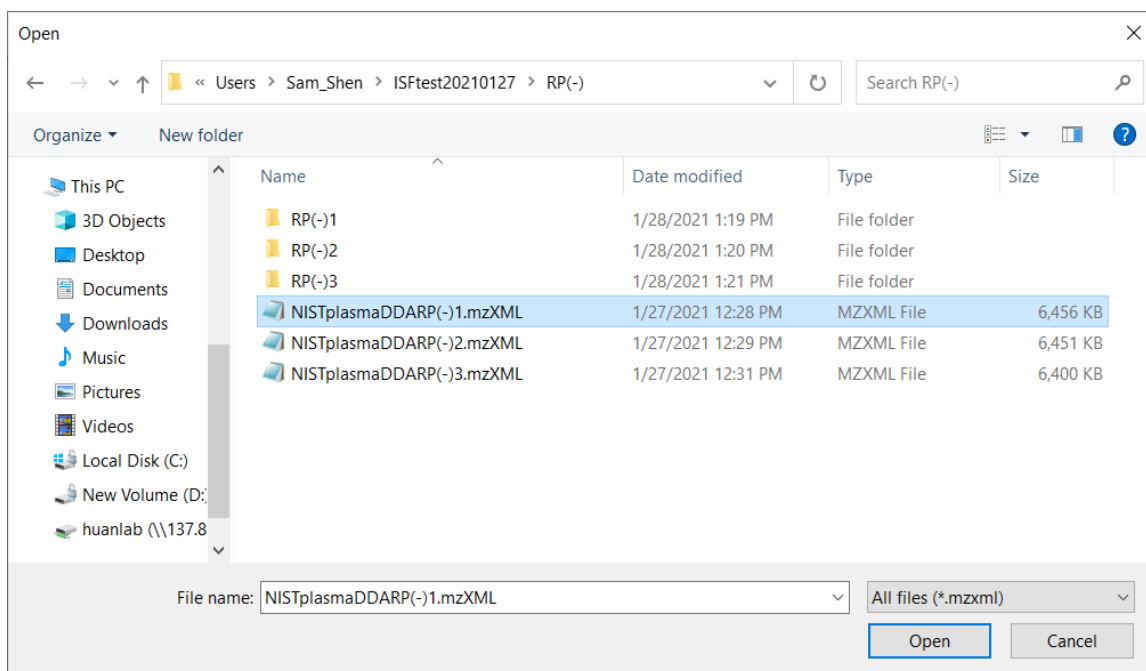


Figure 2.6: Select a single mzXML file.

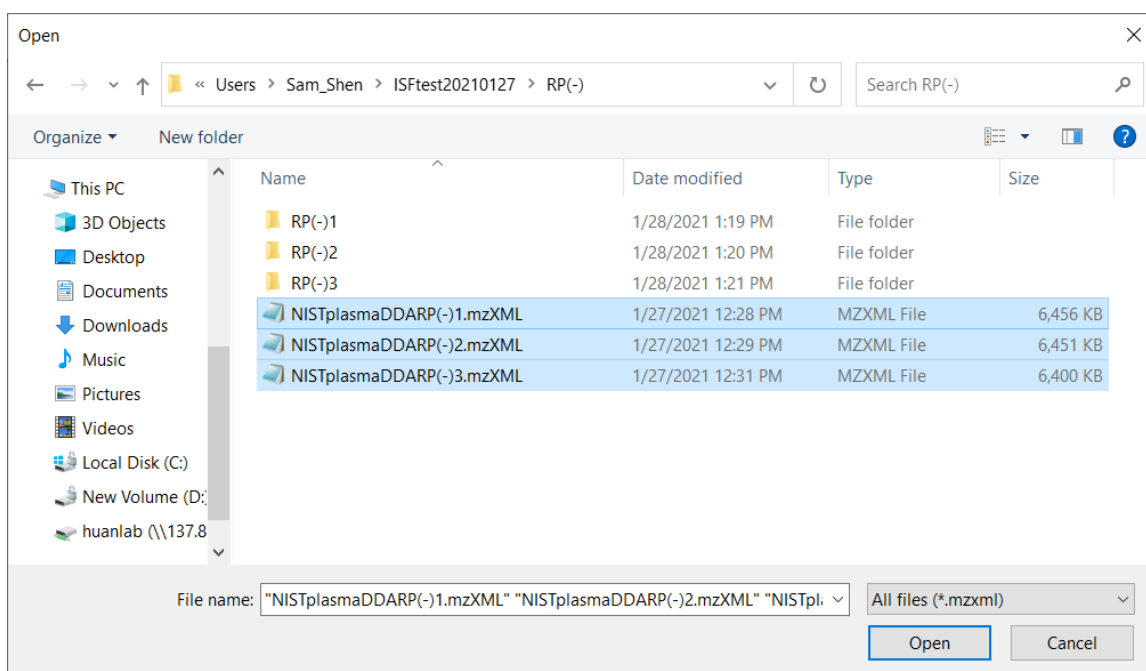


Figure 2.7: Select multiple mzXML files.



2.4.2 Feature Generation

Click on either the “CSV” or “Custom” button to upload a CSV file of features or a customize an XCMS “Centwave” feature extraction workflow, respectively.

If you choose to upload a CSV file, your file must contain only columns in the following order: m/z, retention time, min retention time, max retention time, followed by an additional column containing the intensities of features detected in each sample. Note: column 3 and column 4 are the retention time of the feature edges, and all three columns containing retention time information should be in seconds. Example feature table formats are shown in Figure 2.8 and Figure 2.9 below for single and multi-sample analysis, respectively. After uploading your feature table, EVA will prompt you in a pop-up window to select a smoothing level for plotting chromatograms. We suggest using your sample’s spectral rate as the smoothing level.

	A	B	C	D	E
1	mz	rt	rtmin	rtmax	sample1int
2	44.9987	1445.446	1426.834	1458.288	34256
3	44.99872	1268.429	1220.954	1317.862	39619
4	44.99875	1680.279	1673.369	1694.875	37135
5	46.00216	1680.279	1678.045	1686.285	57531
6	56.99608	54.864	41.499	65.908	30211
7	56.99609	1691.72	1674.253	1702.751	50306
8	59.00222	745.875	740.882	748.46	47749
9	59.08583	1791.71	1789.759	1793.692	43918
10	59.15161	624.157	622.836	627.838	300341
11	59.24167	653.731	651.147	658.42	226883
12	59.26377	1655.052	1652.33	1656.298	40967
13	59.34878	177.422	173.675	180.406	324280
14	59.41398	583.628	582.819	587.602	36172
15	59.46077	1061.042	1056.856	1066.418	45134

Figure 2.8: Example feature table for single sample analysis.



	A	B	C	D	E	F	G
1	mz	rt	rtmin	rtmax	sample1int	sample2int	sample3int
2	44.9987	1445.446	1426.834	1458.288	34256	33006	27923
3	44.99872	1268.429	1220.954	1317.862	39619	47220	42876
4	44.99875	1680.279	1673.369	1694.875	37135	26779	24859
5	46.00216	1680.279	1678.045	1686.285	57531	47055	41204
6	56.99608	54.864	41.499	65.908	30211	40048	38130
7	56.99609	1691.72	1674.253	1702.751	50306	37896	33341
8	59.00222	745.875	740.882	748.46	47749	295286	303804
9	59.08583	1791.71	1789.759	1793.692	43918	225493	224282
10	59.15161	624.157	622.836	627.838	300341	35545	32738
11	59.24167	653.731	651.147	658.42	226883	321182	303804
12	59.26377	1655.052	1652.33	1656.298	40967	31060	28946
13	59.34878	177.422	173.675	180.406	324280	40916	34768
14	59.41398	583.628	582.819	587.602	36172	33976	33206
15	59.46077	1061.042	1056.856	1066.418	45134	38563	34698

Figure 2.9: Example feature table for multi-sample analysis.

If you choose to use a customized XCMS workflow, click the “Custom” button, and EVA will display a pop-up window to set important XCMS feature extraction parameters. See Table 2.1 below for details of each parameter.

Parameter	Usage
ppm	Maximal tolerated m/z deviation in consecutive scans, in ppm (parts per million)
Peak Width	Minimum (first box)/maximum (second box) chromatographic peak width in seconds
mzdiff	Minimum difference in m/z for peaks with overlapping retention times, can be negative to allow overlap
snthresh	Signal/Noise threshold
Integration Method	Integration method. If =1 peak limits are found through descent on the mexican hat filtered data, if =2 the descent is done on the real data. Method 2 is very accurate but prone to noise, while method 1 is more robust to noise but less exact.
Prefilter	Prefilter step for the first phase. Mass traces are only retained if they contain at least



	[prefilter peaks (first box)] peaks with intensity \geq [prefilter intensity (second box)]
Noise	Useful for data that was centroided without any intensity threshold, centroids with intensity $<$ noise are omitted from ROI detection
Smoothing Level	Set smoothing level for plotting chromatograms. It is suggested to use the sample's spectral rate as the smoothing level.

Table 2.1: Parameters for XCMS feature extraction.

2.4.3 Feature Evaluation Model

Under “Feature Evaluation Model”, click “Default” to use the default deep learning CNN model included in EVA. Otherwise, click “Custom” to upload your own CNN model.

2.5 Run

Click the “Run” button to start feature evaluation process. If you did not upload one or more required file before clicking on “Run”, the progress circles (1, 2, or 3) appears red along with an error message. You should then upload all the necessary files and click “Run” again. If the Python or R exe files are not selected, an error message will appear prompting you to do so. Once the processing finishes, EVA will automatically display the results on the middle and right panels.



3 Results Visualization & Export

3.1 View Results

The middle panel shows the feature table with prediction results. Click on any row to show the corresponding chromatogram. Click on any of the column headers to sort the feature table in descending or ascending order. The right panel summarizes the prediction results in both bar and pie charts. An illustrated schematic diagram is shown in Figure 3.1 below.

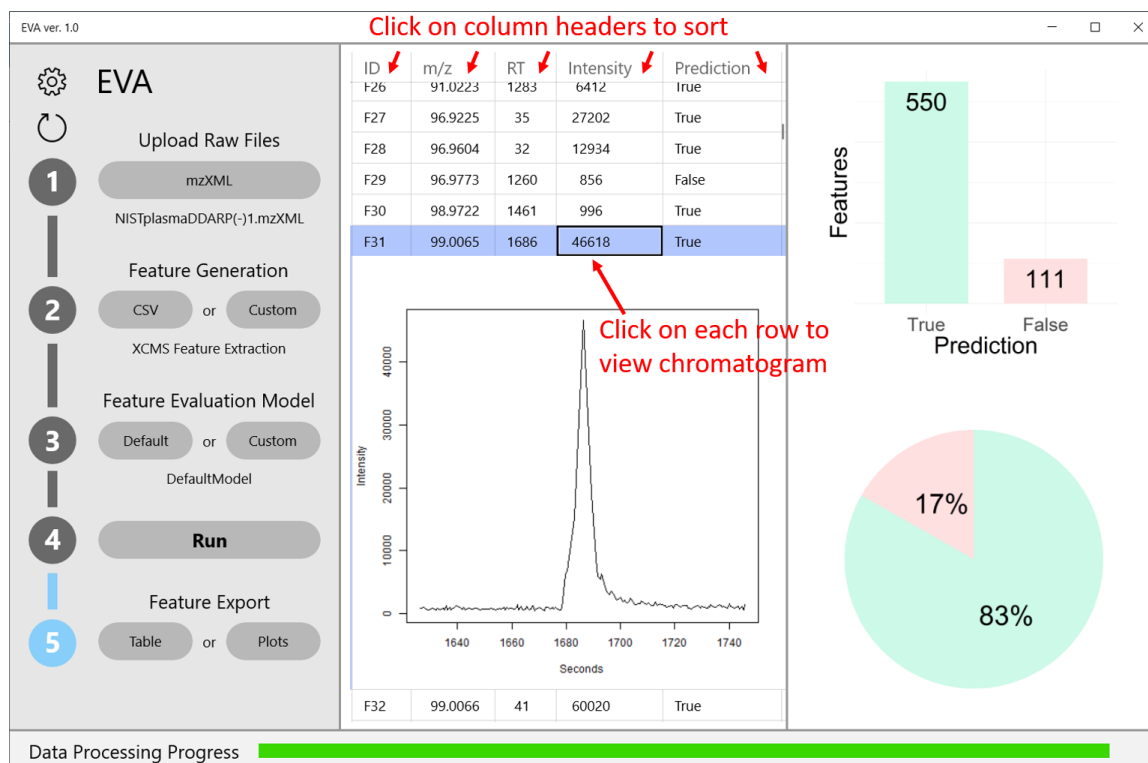


Figure 3.1: EVA results page.

3.2 Download Results

Download the feature table with prediction results as a CSV file by clicking the “Table” button at the bottom of the left panel. Download all chromatograms by clicking the “Plots” button.

3.3 Restart

Start a new session by clicking on the refresh button on the top of the left panel.