featureHunter

The Ultimate Data Analysis Solution for ADDUCTOMICS

User manual for

FeatureHunter ver.1.4

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About *FeatureHunter*

FeatureHunter was developed as the ultimate solution for untargeted nucleic acid adductomics, empowering researchers to effortlessly process data from a wide range of cutting-edge high-resolution mass spectrometry and leading MS vendors (e.g., Agilent, Thermo, and Waters).

featureHunter
The Ultimate Data Analysis Solution for ADDUCTOMICS

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1. System Requirements

Recommended requirements

- 4 or more CPU cores
- 16 GB or more RAM
- 64-bit Windows 10 or later (macOS is not available)

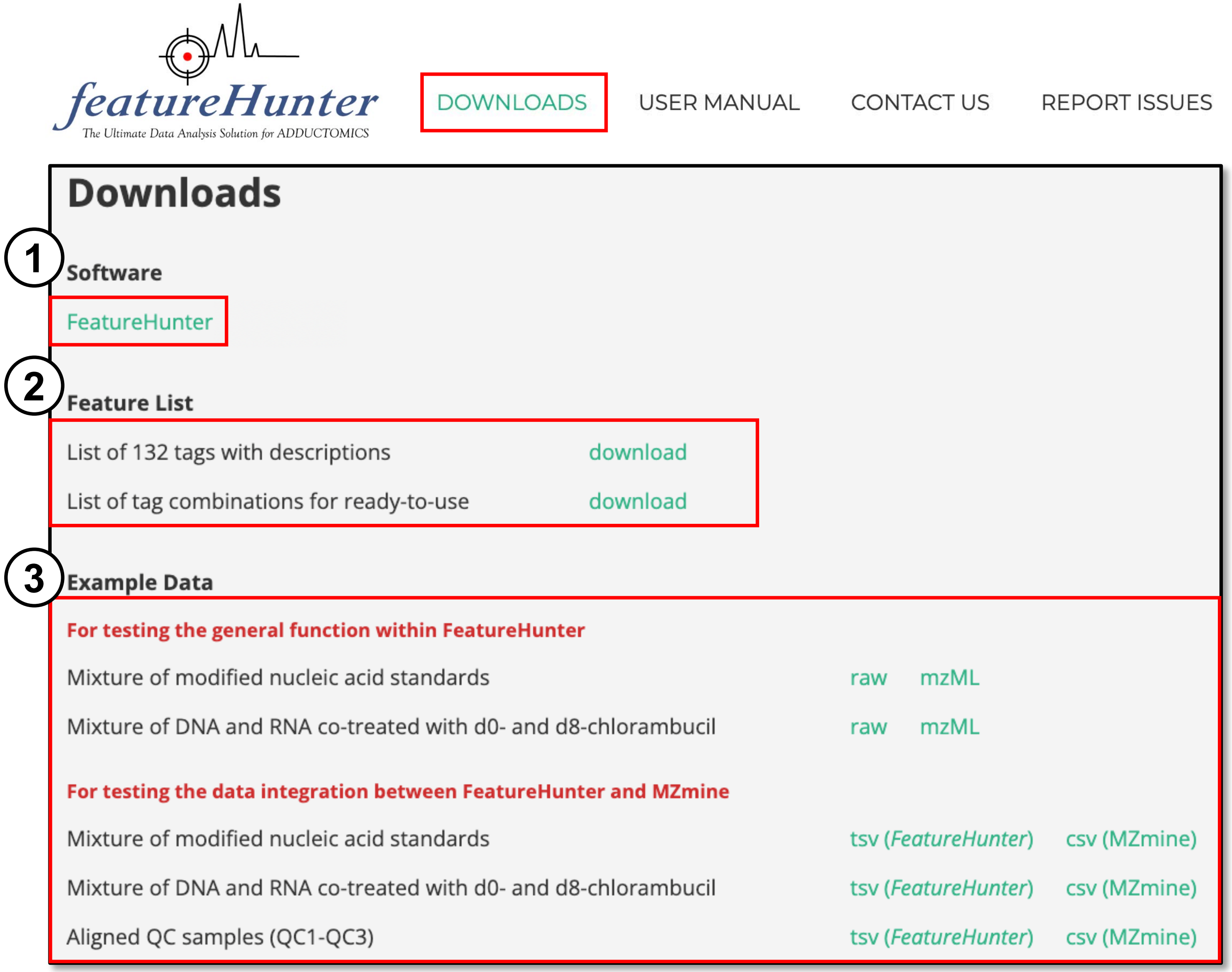
2. Installation

Prerequisites

- Visual C++ redistributable package (64-bit)
- .NET Framework 4.7.2 or later (64-bit)
- FeatureHunter (ver. 1.4)
- MSConvert

- The successful running of *FeatureHunter* requires the installation of Visual C++ redistributable package and .NET Framework.
- A detailed guide for the installation of Visual C++ redistributable package and .NET Framework can be found in **Appendix A1 and A2**.
- The raw data obtained from vendor format must be converted into .mzML format (please see **Appendix A3**) for successfully running in *FeatureHunter.*

Download Software, Feature List and Example Data



Downloads

Software

FeatureHunter

Feature List

List of 132 tags with descriptions	download
List of tag combinations for ready-to-use	download

Example Data

For testing the general function within FeatureHunter

Mixture of modified nucleic acid standards	raw	mzML
Mixture of DNA and RNA co-treated with d0- and d8-chlorambucil	raw	mzML

For testing the data integration between FeatureHunter and MZmine

Mixture of modified nucleic acid standards	tsv (*FeatureHunter*)	csv (MZmine)
Mixture of DNA and RNA co-treated with d0- and d8-chlorambucil	tsv (*FeatureHunter*)	csv (MZmine)
Aligned QC samples (QC1-QC3)	tsv (*FeatureHunter*)	csv (MZmine)

Fig-1. The page for downloading the software, the list of tag descriptions and applications, and example data

***FeatureHunter can be downloaded from the following link*:**

https://msomics.abrc.sinica.edu.tw/FeatureHunter/?page_id=9

① Download the **Software**

② Download the **Feature List**, which guides users to choose the tags (features) for extracting compounds of interest

③ Download the **Example Data**, available in various formats (Thermo.raw and mzML) and outputs (.tsv and .csv), to freely test and verify the software

Checking the download file

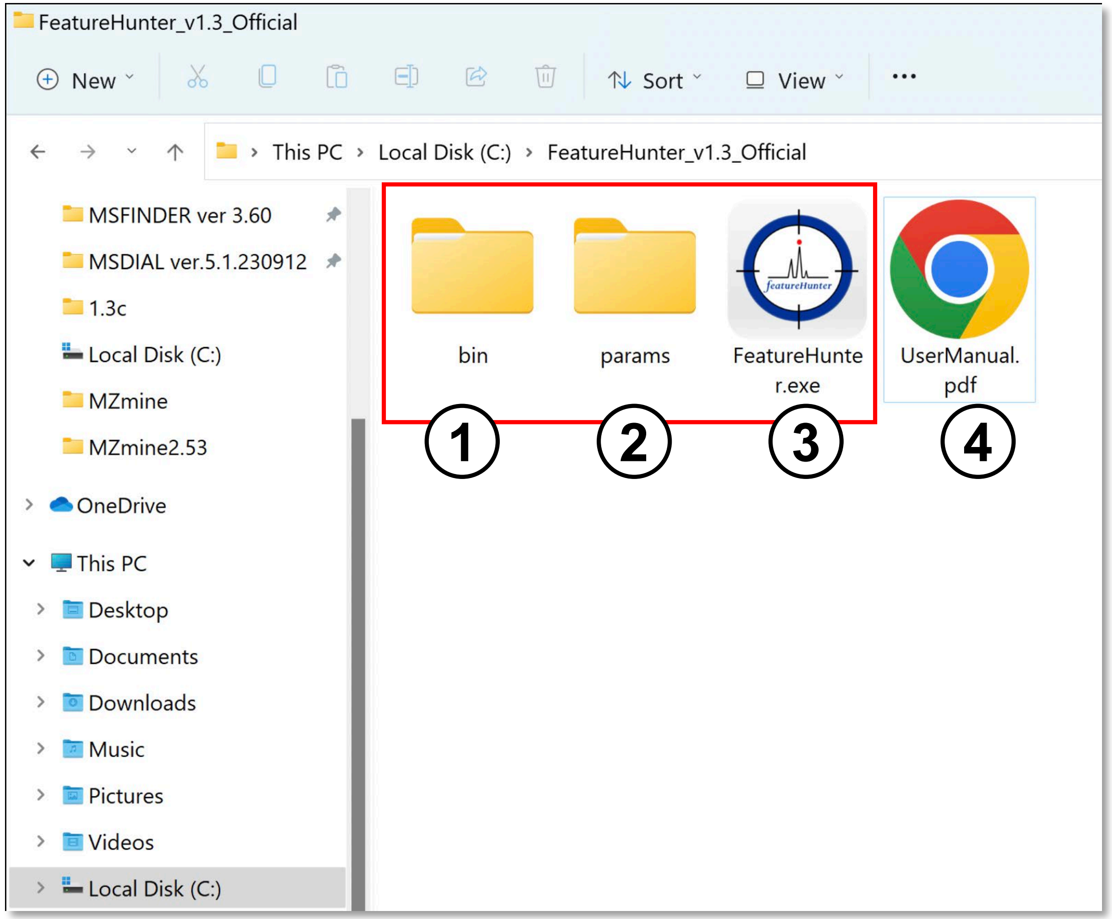


Fig-2. The items included in the *FeatureHunter* download folder

Once the download is complete, the folder will contain the following four items:

① **bin:** This folder contains the executable files of *FeatureHunter*.

② **params:** This folder contains two important parameter files used in *FeatureHunter*: one for feature extraction settings and another for artificial adducts match configurations.

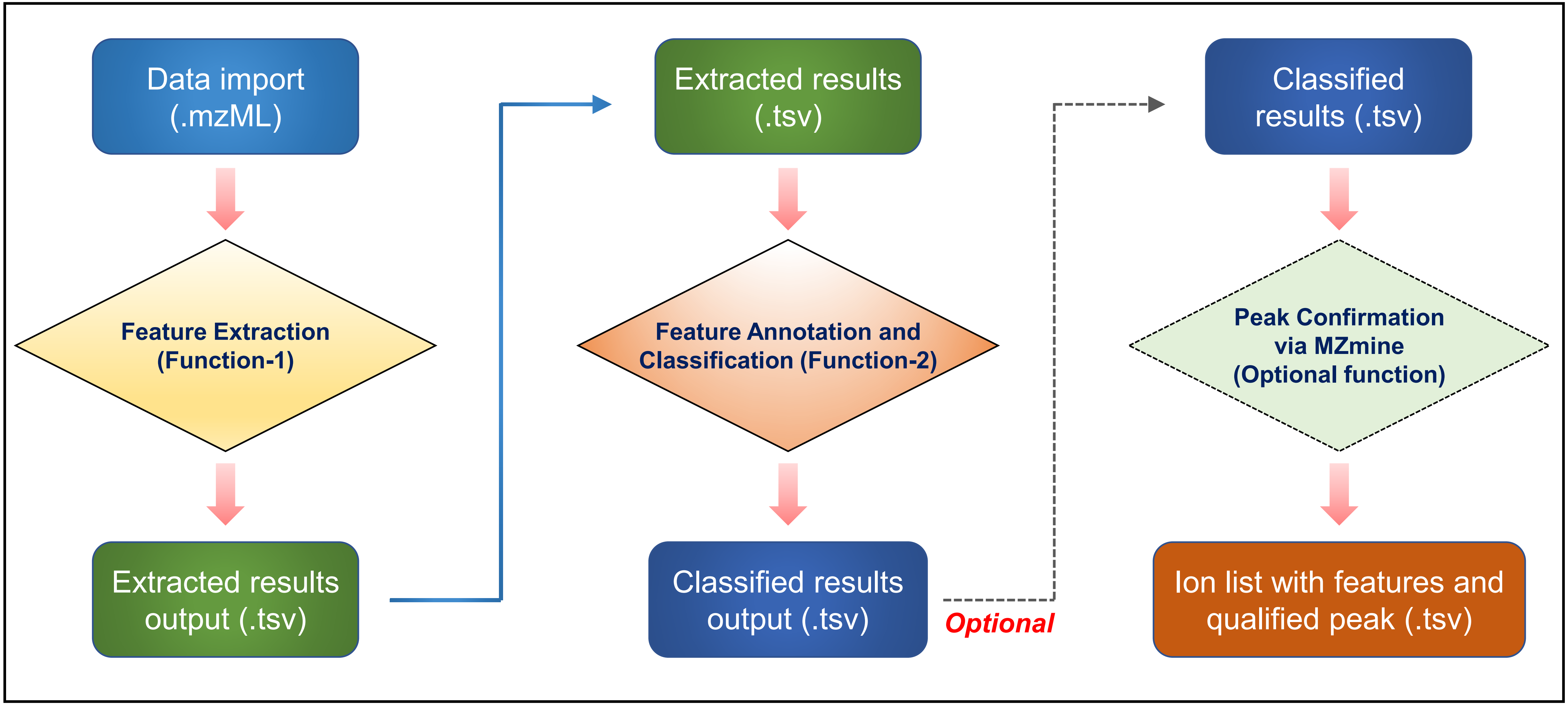
③ **FeatureHunter.exe:** The main program executable for *FeatureHunter*.

④ **UserManual.pdf:** A tutorial providing a guidance on how to use *FeatureHunter*.

- For successful execution of *FeatureHunter*, place the **(1)** “bin” folder, **(2)** “params” folder, and **(3)** “FeatureHunter.exe” file in the same directory.

- Additionally, it is recommended to store the downloaded folder on the C drive at the path: **C:\FeatureHunter**

3. Steps for the data processing within *FeatureHunter*



Data import (.mzML)

Feature Extraction (Function-1)

Extracted results output (.tsv)

Extracted results (.tsv)

Feature Annotation and Classification (Function-2)

Classified results output (.tsv)

Optional

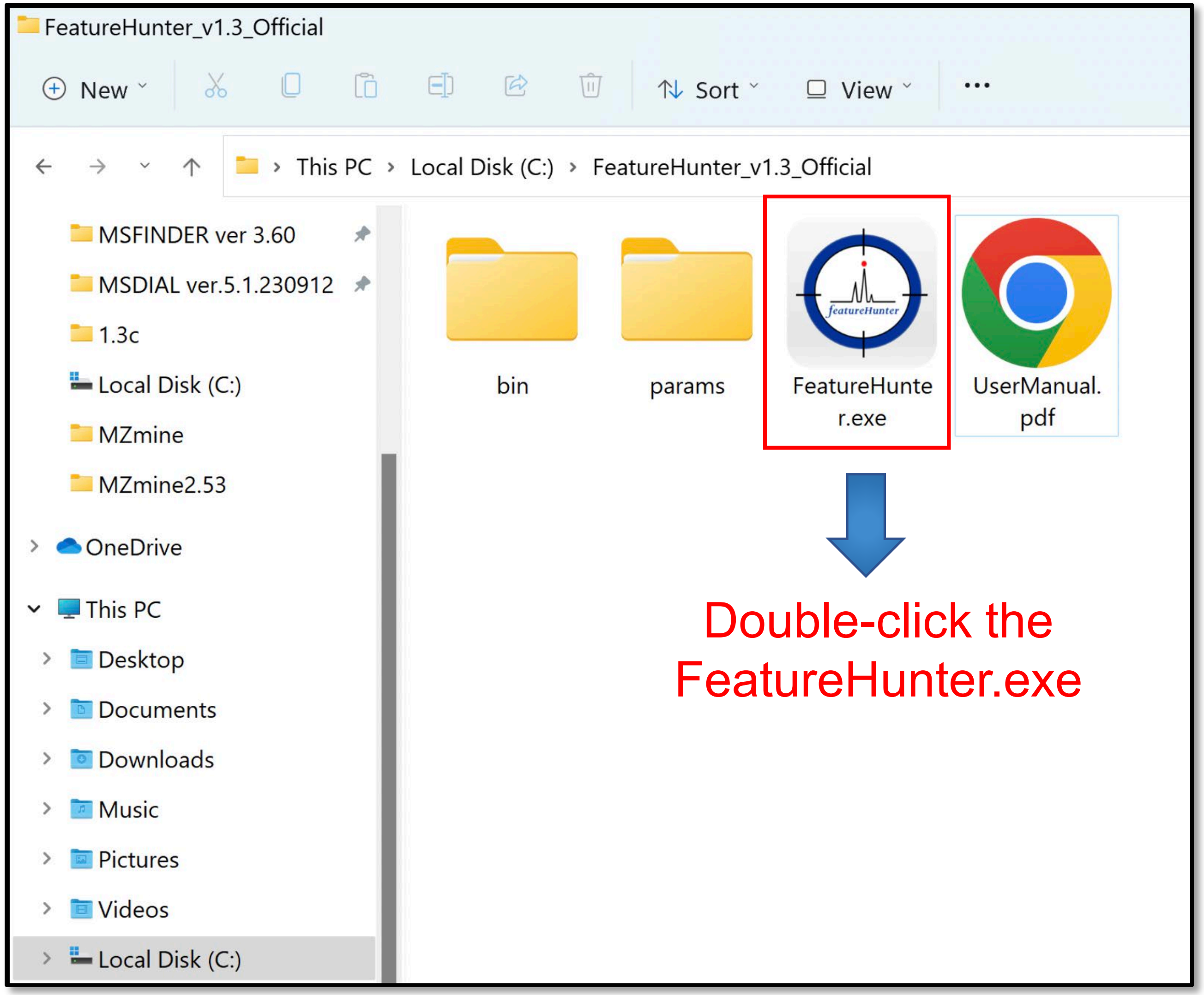
Classified results (.tsv)

Peak Confirmation via MZmine (Optional function)

Ion list with features and qualified peak (.tsv)

Fig-3. The flowchart of the *FeatureHunter* processing

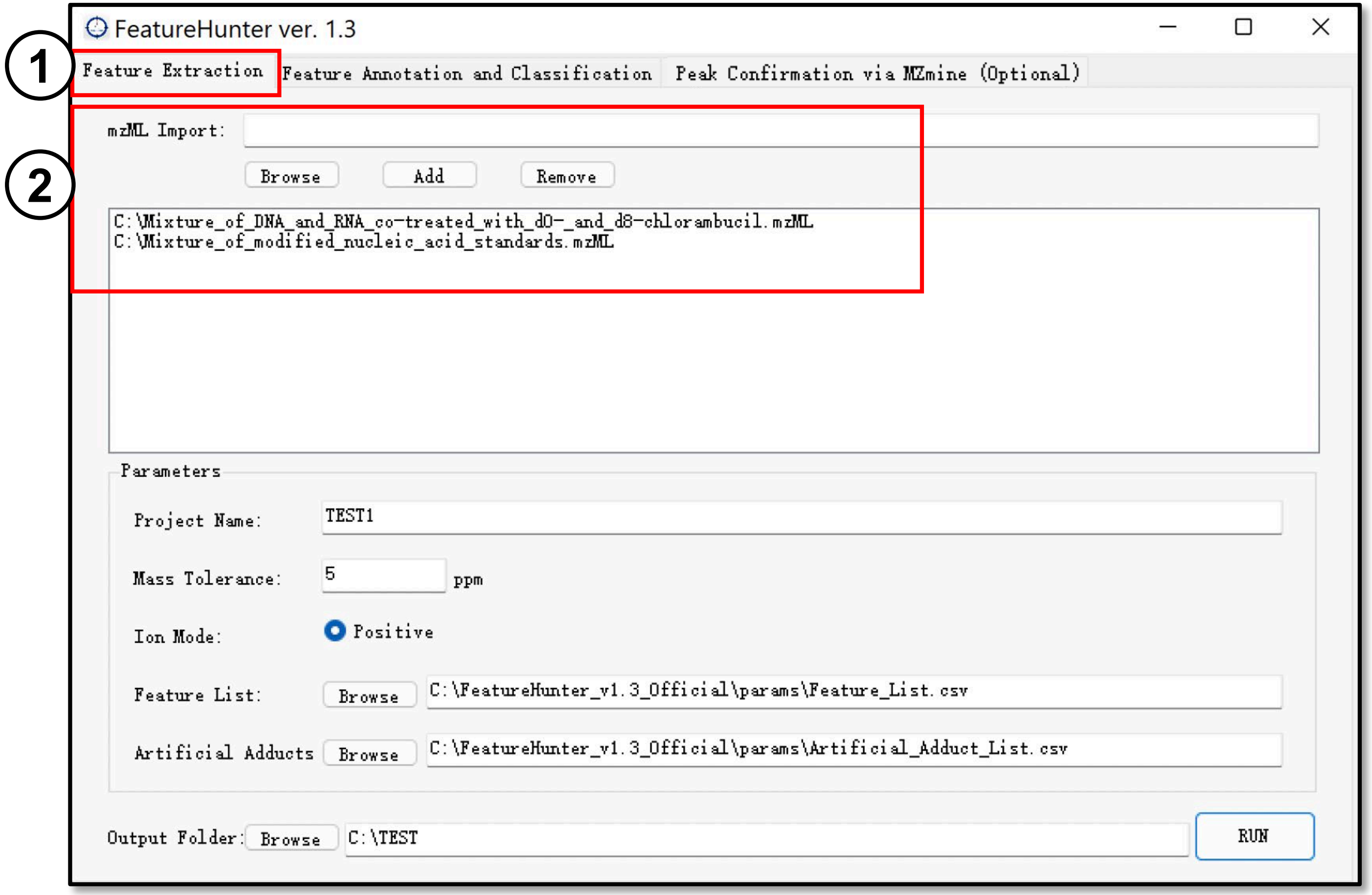
3-1. Launch the FeatureHunter **[Step-1]**



Double-click the FeatureHunter.exe

Fig-4. Starting the software

3-2. Feature extraction (Function-1) **[Step-2]**



mzML Import:

C:\Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.mzML
C:\Mixture_of_modified_nucleic_acid_standards.mzML

Project Name: TEST1

Mass Tolerance: 5 ppm

Ion Mode: ● Positive

Feature List: Browse C:\FeatureHunter_v1.3_Official\params\Feature_List.csv

Artificial Adducts Browse C:\FeatureHunter_v1.3_Official\params\Artificial_Adduct_List.csv

Output Folder: Browse C:\TEST

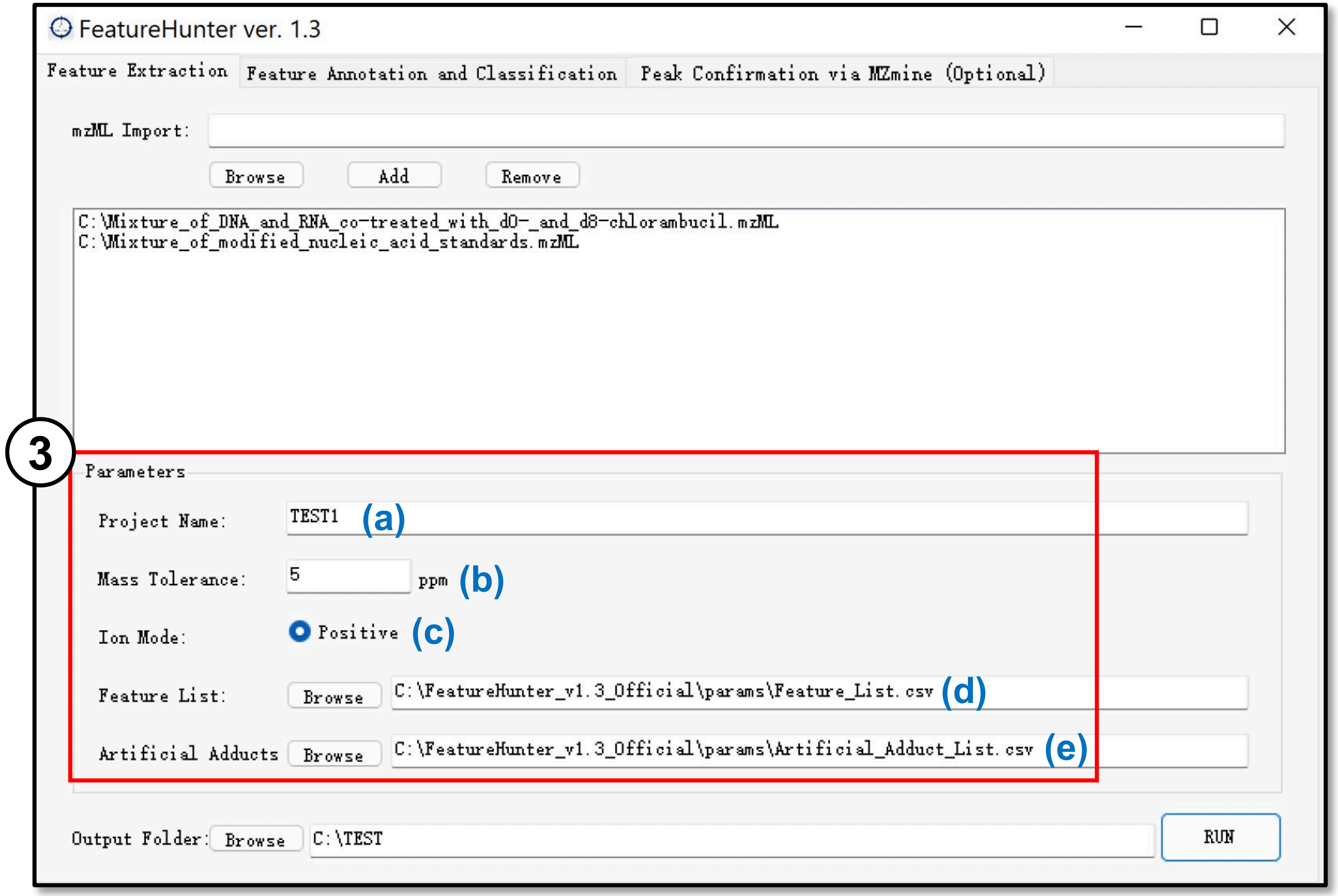
Fig-5. Interface of “Feature Extraction” in *FeatureHunter*

① Select the “Feature Extraction”.

② Click “Browse” to import the raw data file in **mzML** format.

As the mzML format was required, carefully refer to the parameter settings for data conversion in MSconvert provided in **Appendix A3**.

3-2. Feature extraction (Function-1) [Step-2]



mzML Import:

C:\Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.mzML
C:\Mixture_of_modified_nucleic_acid_standards.mzML

Project Name: TEST1
Mass Tolerance: 5 ppm
Ion Mode: ● Positive
Feature List: C:\FeatureHunter_v1.3_Official\params\Feature_List.csv
Artificial Adducts: C:\FeatureHunter_v1.3_Official\params\Artificial_Adduct_List.csv
Output Folder: C:\TEST

Fig-5. Interface of “Feature Extraction” in *FeatureHunter*

③ Set the parameters:

(a) Specify a "Project Name" which will be automatically added as a prefix to the output file name. For example: **TEST1**_file name.

(b) Set the “Mass Tolerance” with ppm for the artificial adduct match and the peak height of precursor’s EIC search.

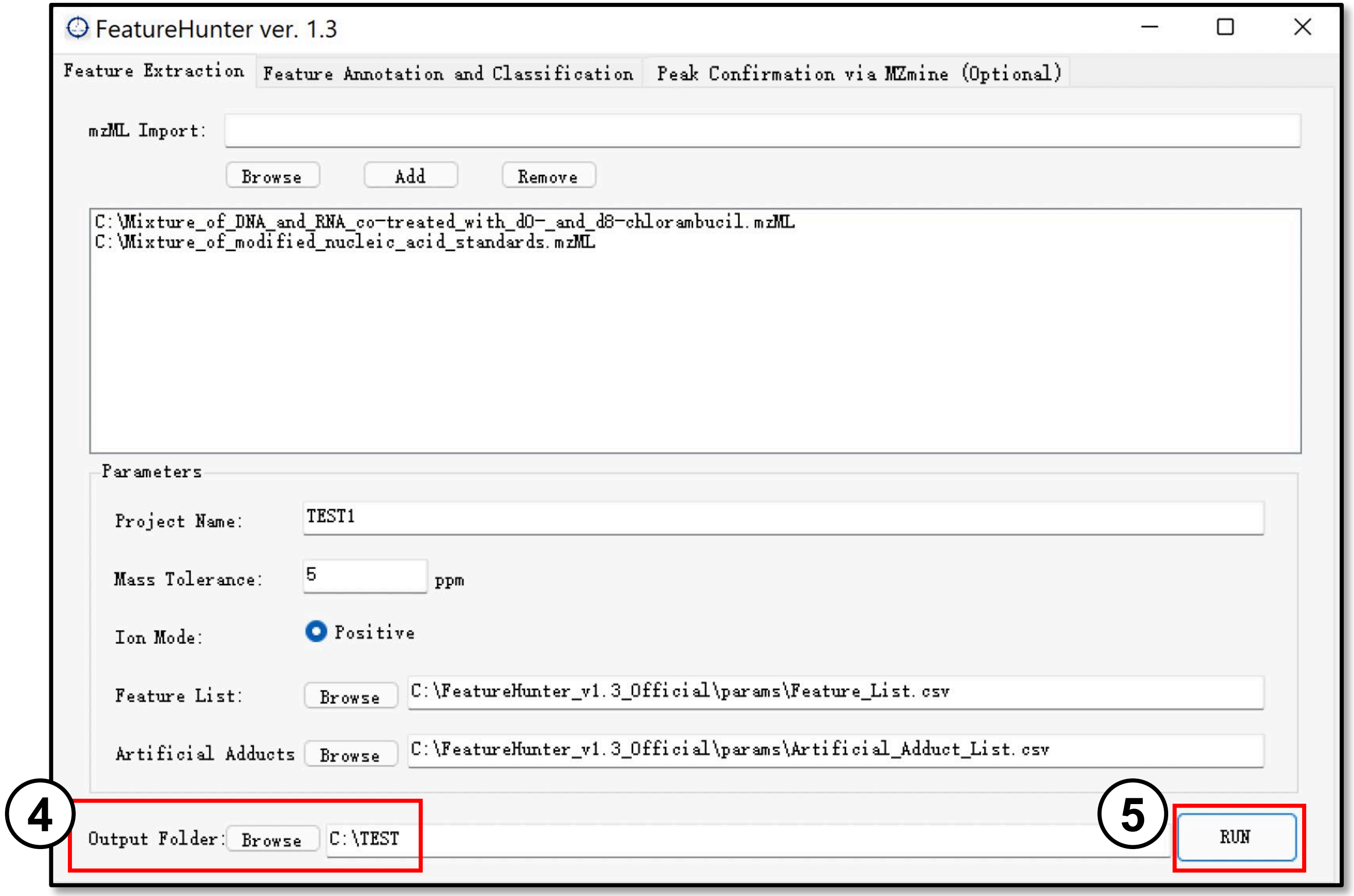
(c) Positive ion mode is default setting.

(d) Import the Feature List file. (The default Feature List.CSV can be found in the params folder)

(e) Import the Artificial Adducts List file. (The default Artificial Adducts List.CSV can be found in the params folder)

The Feature List and Artificial Adducts List can be edited and are detailed in **Appendix A4 and A5**, respectively.

3-2. Feature extraction (Function-1) **[Step-2]**



mzML Import:

C:\Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.mzML
C:\Mixture_of_modified_nucleic_acid_standards.mzML

Parameters

Project Name: TEST1

Mass Tolerance: 5 ppm

Ion Mode: ● Positive

Feature List: C:\FeatureHunter_v1.3_Official\params\Feature_List.csv

Artificial Adducts C:\FeatureHunter_v1.3_Official\params\Artificial_Adduct_List.csv

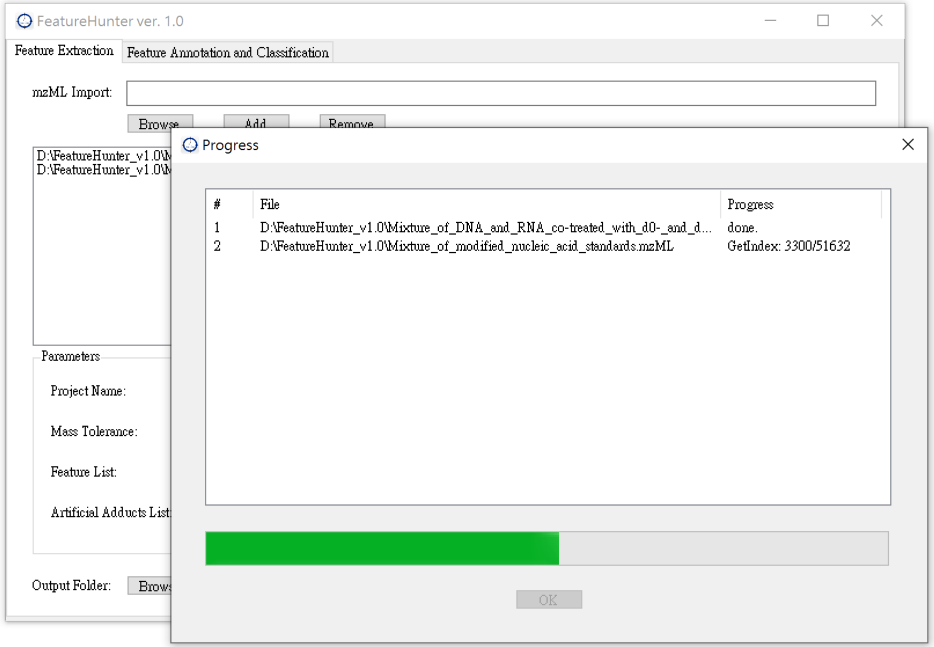
Output Folder: C:\TEST

Fig-5. Interface of “Feature Extraction” in *FeatureHunter*

④ Specify the output path and folder. (The output files will be in .TSV format.)

⑤ Click "RUN" to start execution.

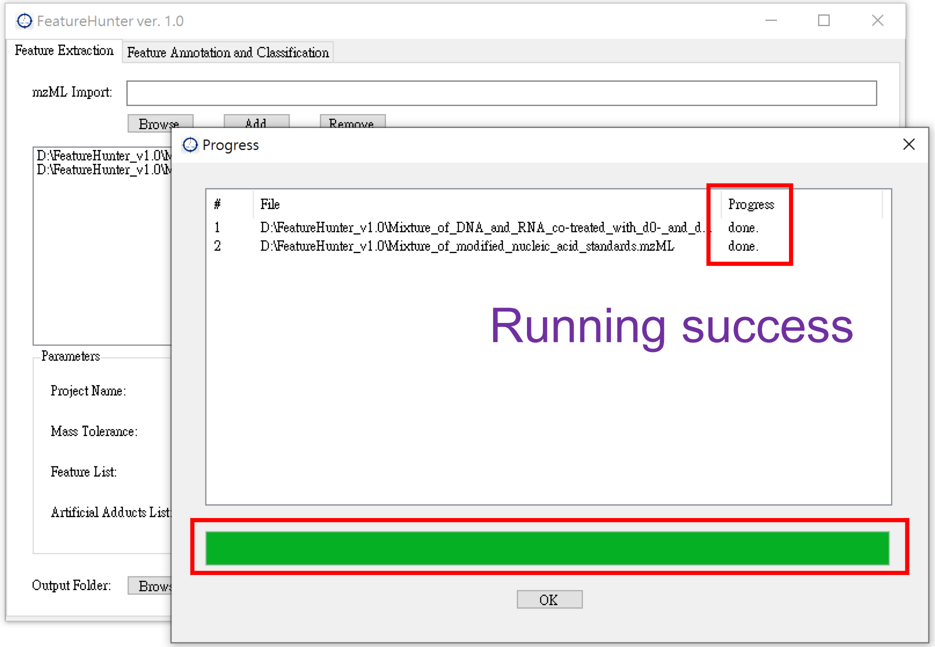
When processing



#	File	Progress
1	D:\FeatureHunter_v1.0\Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d...	done.
2	D:\FeatureHunter_v1.0\Mixture_of_modified_nucleic_acid_standards.mzML	GetIndex: 3300/51632

Fig-6. Display of the running progress during the execution

Finished



#	File	Progress
1	D:\FeatureHunter_v1.0\Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d...	done.
2	D:\FeatureHunter_v1.0\Mixture_of_modified_nucleic_acid_standards.mzML	done.

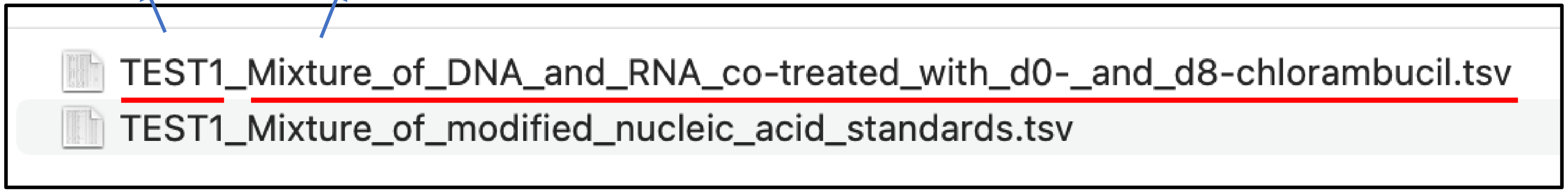
Running success

Fig-7. Display of the running completed

Project name

Data file name

Output success

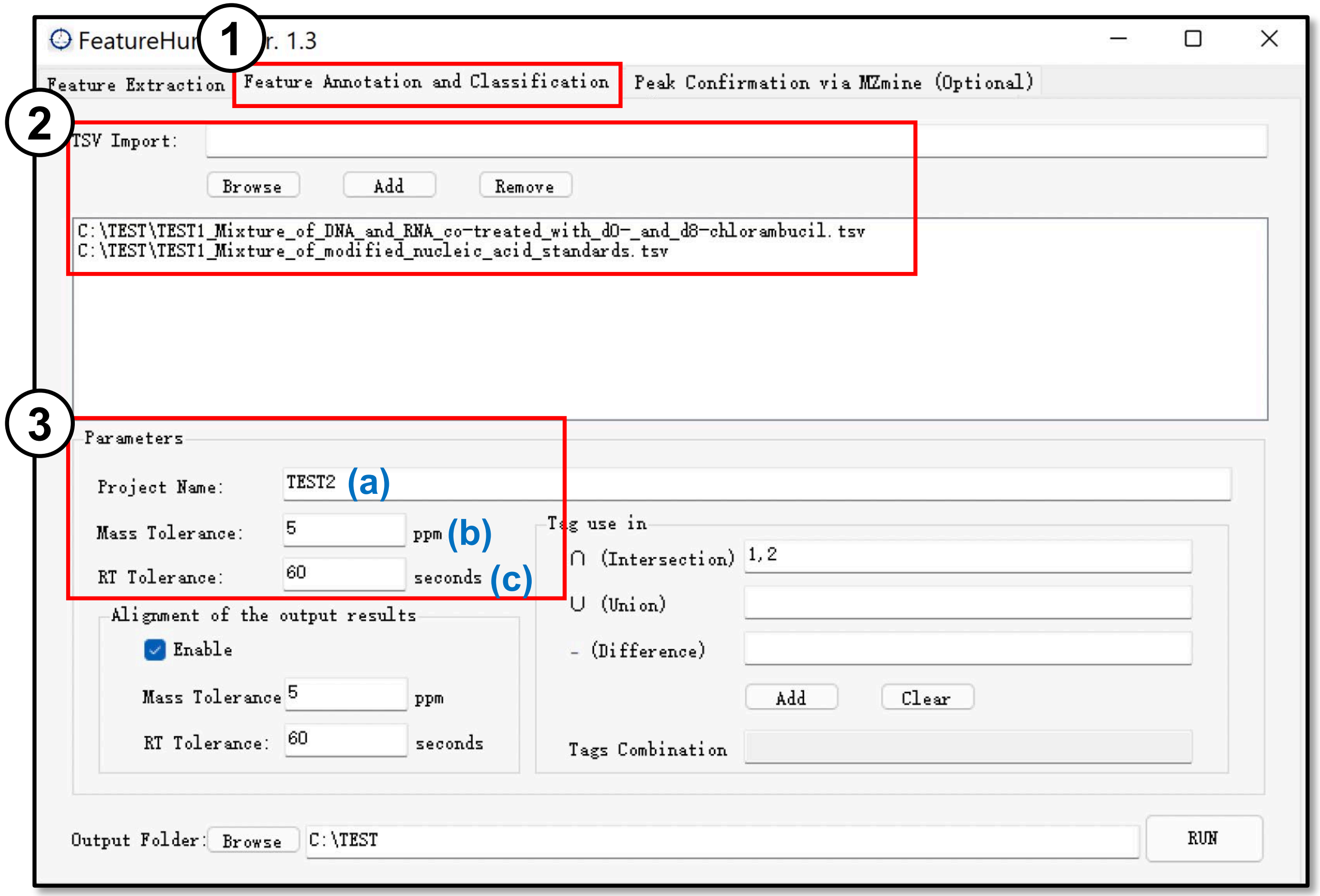


TEST1_Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.tsv

TEST1_Mixture_of_modified_nucleic_acid_standards.tsv

Fig-8. The results output in the specified output folder

3-3. Feature Annotation and Classification (Function-2) [Step-3]



TSV Import:

C:\TEST\TEST1_Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.tsv
C:\TEST\TEST1_Mixture_of_modified_nucleic_acid_standards.tsv

Project Name: TEST2
Mass Tolerance: 5 ppm
RT Tolerance: 60 seconds

☑ Enable
Mass Tolerance 5 ppm
RT Tolerance: 60 seconds

∩ (Intersection) 1,2
∪ (Union)
- (Difference)

Output Folder: C:\TEST

Fig-9. Interface of “Feature Annotation and Classification” in *FeatureHunter*

① Select the “Feature Annotation and Classification”.

② Click “Browse” to import the .TSV file that was generated from Feature Extraction (Function-1).

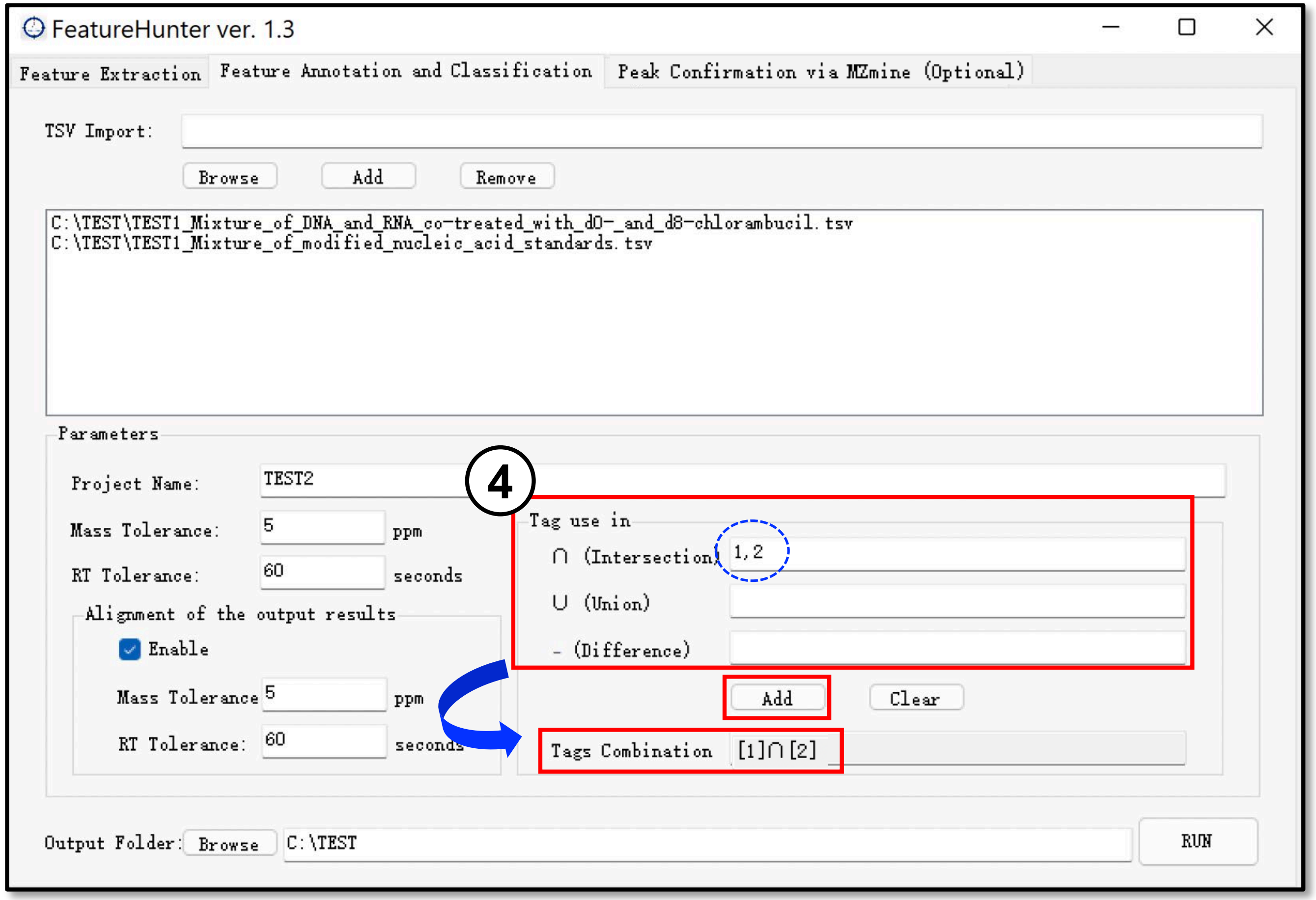
③ Set the parameters:

(a) Specify a "Project Name" which will be automatically added as a prefix to the output file name. For example: **TEST2**_TEST1_file name.

(b) Set the “Mass Tolerance” with ppm for the feature classification.

(c) Set the “RT Tolerance” with seconds for the feature classification.

3-3. Feature Annotation and Classification (Function-2) [Step-3]



TSV Import:

C:\TEST\TEST1_Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.tsv
C:\TEST\TEST1_Mixture_of_modified_nucleic_acid_standards.tsv

Project Name: TEST2
Mass Tolerance: 5 ppm
RT Tolerance: 60 seconds

Alignment of the output results
☑ Enable
Mass Tolerance 5 ppm
RT Tolerance: 60 seconds

Tag use in
∩ (Intersection) 1, 2
∪ (Union)
- (Difference)

Tags Combination [1]∩[2]

Output Folder: C:\TEST

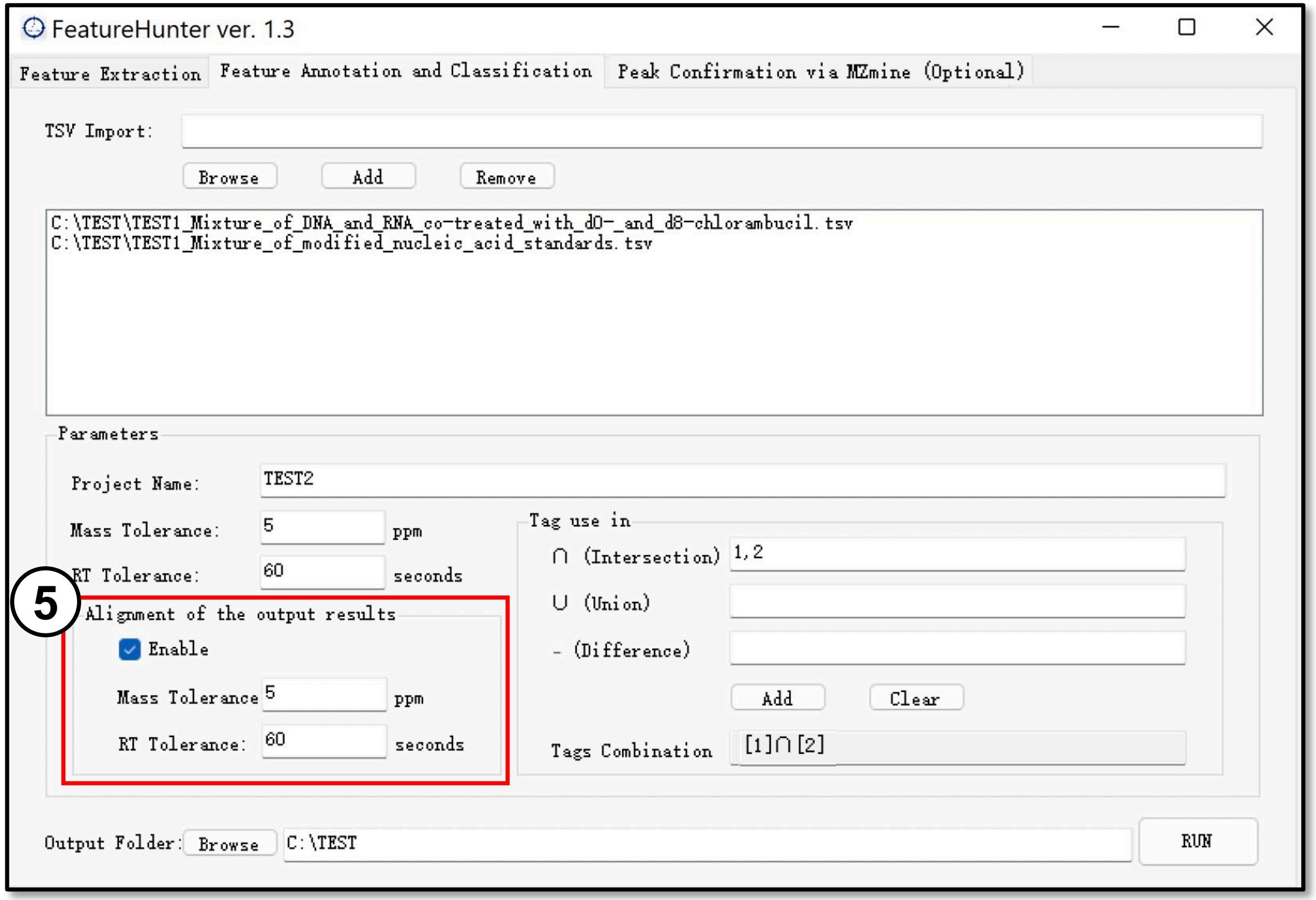
Fig-9. Interface of “Feature Annotation and Classification” in *FeatureHunter*

④ Use tags to classify the annotated features.

- The tags that can be used correspond to the Feature List.csv file located in the params folder.
- The identity of each tag is described in ’List of 132 tags with descriptions’ which can be downloaded from website.
- Tags can be inputted individually or in combination using the **intersection (∩)**, **union (∪)**, or **difference (−)** functions.
- Multiple tags can be inputted by separating them with a comma.
- Leaving the field blank is allowed if the function is not being used.

The guide on tag usage can be found in **Appendix A6**.

3-3. Feature Annotation and Classification (Function-2) **[Step-3]**



TSV Import:

C:\TEST\TEST1_Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.tsv
C:\TEST\TEST1_Mixture_of_modified_nucleic_acid_standards.tsv

Project Name: TEST2
Mass Tolerance: 5 ppm
RT Tolerance: 60 seconds

Alignment of the output results
☑ Enable
Mass Tolerance 5 ppm
RT Tolerance: 60 seconds

∩ (Intersection) 1,2
∪ (Union)
- (Difference)
Tags Combination [1]∩[2]

Output Folder: C:\TEST

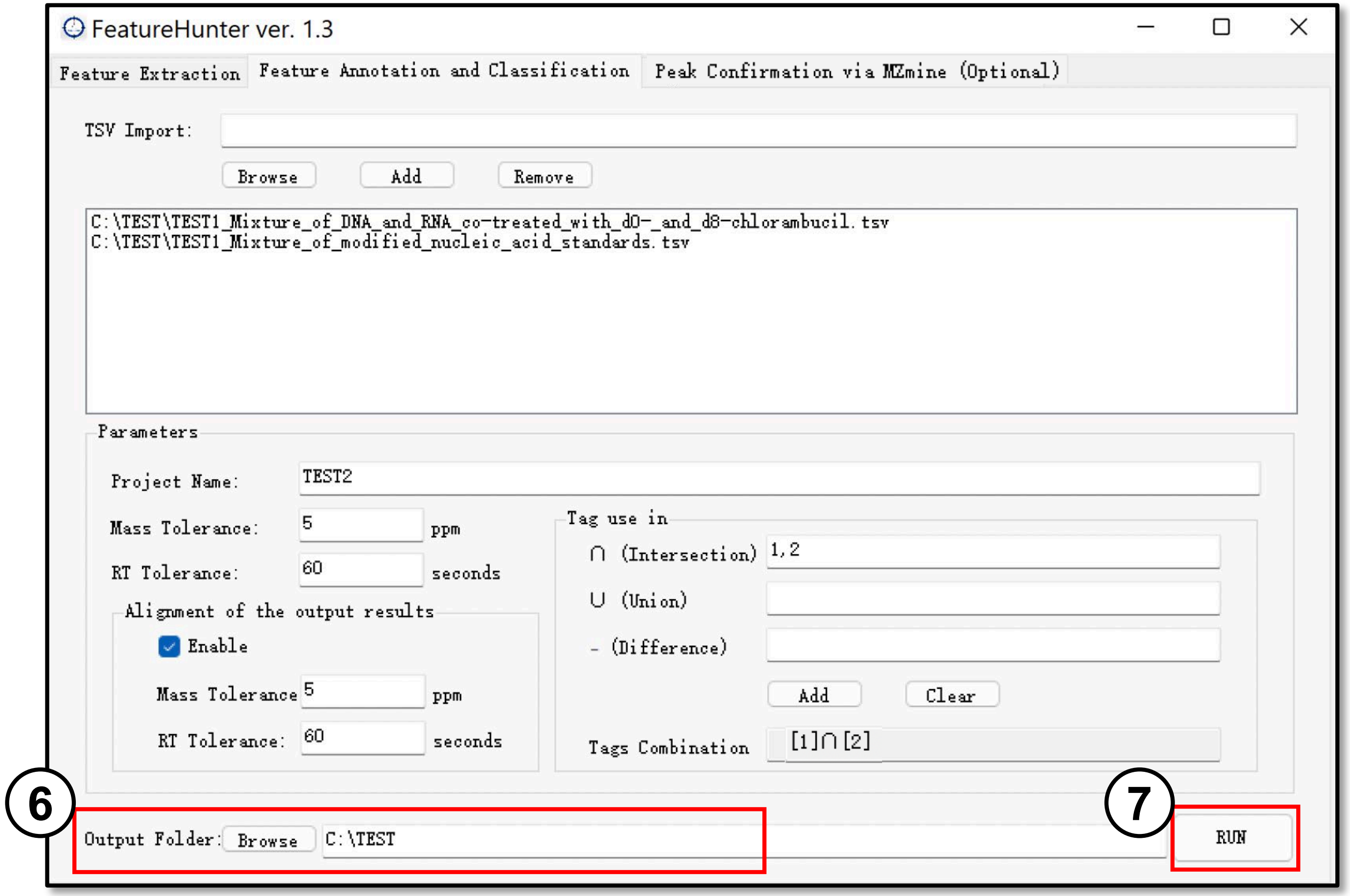
Fig-9. Interface of “Feature Annotation and Classification” in *FeatureHunter*

⑤ **(Optional)** Alignment of the output results.

- At least two .TSV file inputs are required for alignment.
- Enabling this option means that the classified results are generated from multiple sample analyses, and they are further aligned to create an aligned feature peak list with precursor ion m/z, retention time (RT), and peak intensity.
- Set the “Mass Tolerance” in ppm to align the ion signals across different datasets.
- Set the “RT Tolerance” in seconds to align the ion signals across different datasets based on their RT.

- Signal alignment was initiated using the m/z and RT values obtained from importing the first dataset (.tsv).

- The notation ‘n/a’ indicated the absence of aligned signals in the current data.



TSV Import:

C:\TEST\TEST1_Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.tsv
C:\TEST\TEST1_Mixture_of_modified_nucleic_acid_standards.tsv

Parameters

Project Name: TEST2

Mass Tolerance: 5 ppm

RT Tolerance: 60 seconds

Alignment of the output results

☑ Enable

Mass Tolerance 5 ppm

RT Tolerance: 60 seconds

Tag use in

∩ (Intersection) 1,2

∪ (Union)

- (Difference)

Tags Combination [1]∩[2]

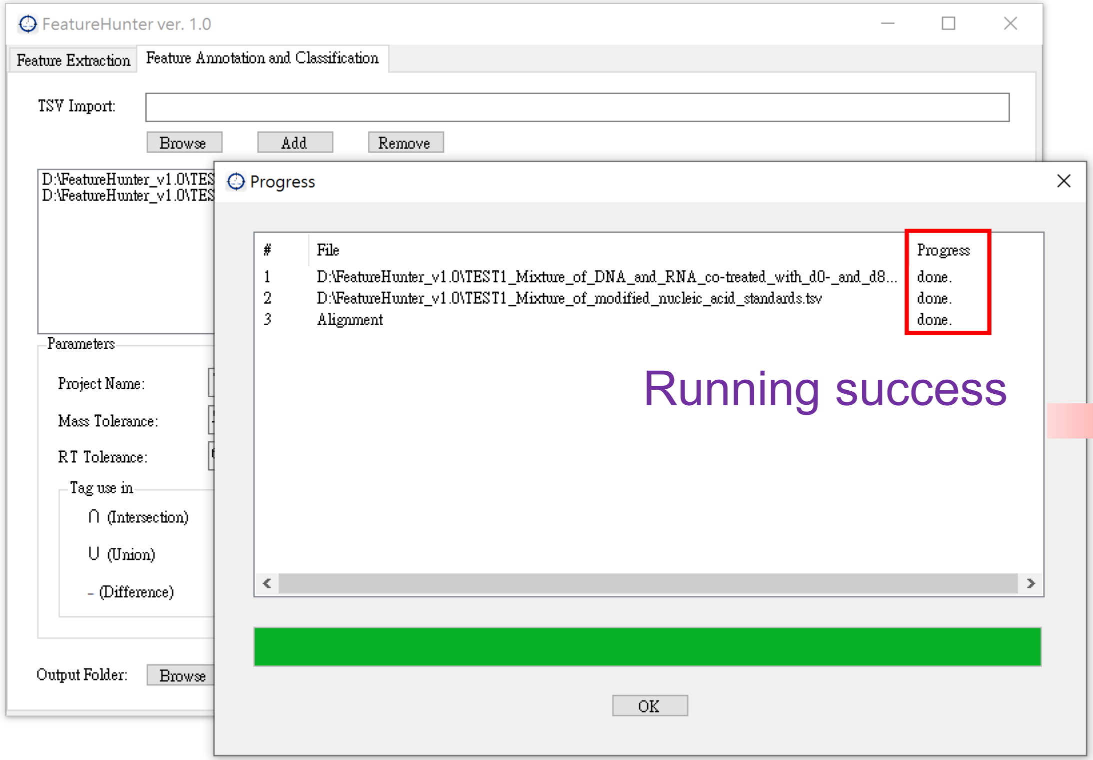
Output Folder: C:\TEST

Fig-9. Interface of “Feature Annotation and Classification” in *FeatureHunter*

⑥ Specify the output path and folder. (The output files will be in .TSV format.)

⑦ Click "RUN" to start execution.

Finished



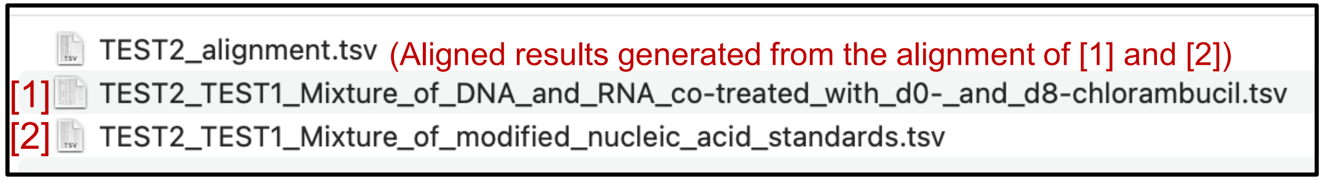
D:\FeatureHunter_v1.0\TES
D:\FeatureHunter_v1.0\TES

#	File	Progress
1	D:\FeatureHunter_v1.0\TEST1_Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8...	done.
2	D:\FeatureHunter_v1.0\TEST1_Mixture_of_modified_nucleic_acid_standards.tsv	done.
3	Alignment	done.

Running success

Fig-10. Display of the running completed

Output success



TEST2_alignment.tsv (Aligned results generated from the alignment of [1] and [2])
[1] TEST2_TEST1_Mixture_of_DNA_and_RNA_co-treated_with_d0-_and_d8-chlorambucil.tsv
[2] TEST2_TEST1_Mixture_of_modified_nucleic_acid_standards.tsv

Fig-11. The individual classified peak list (i.e., [1] and [2]) as well as the aligned results, which are output in the specified output folder

An example of output results from feature annotation and classification (Format-1)

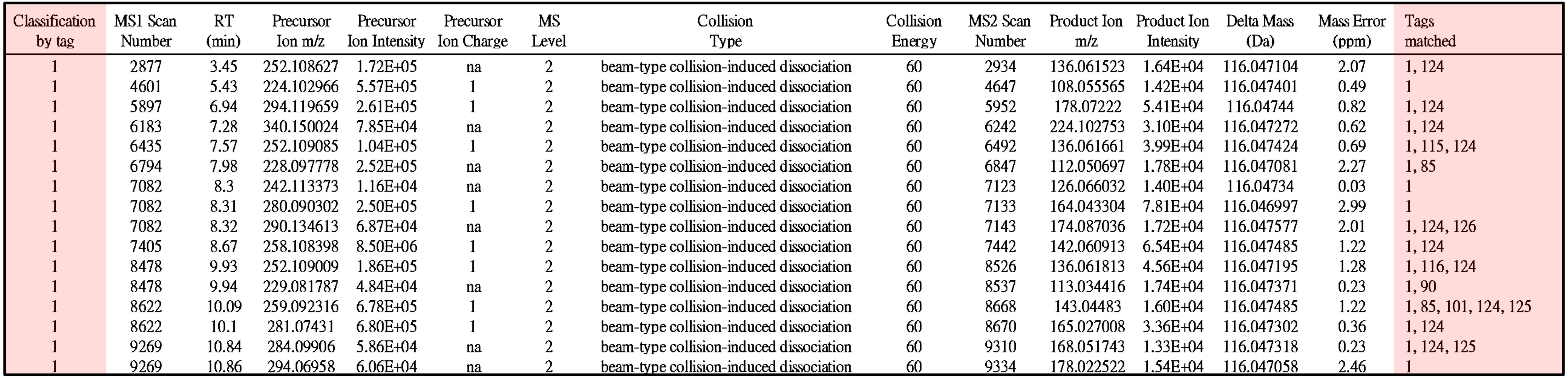
- The output results can be viewed in excel

When using a tag with **category 1 or 2** (see Appendix A4)

Information of detected MS^1 ions

Information of detected MS^2 ions

Information of detected features



Classification by tag	MS1 Scan Number	RT (min)	Precursor Ion m/z	Precursor Ion Intensity	Precursor Ion Charge	MS Level	Collision Type	Collision Energy	MS2 Scan Number	Product Ion m/z	Product Ion Intensity	Delta Mass (Da)	Mass Error (ppm)	Tags matched
1	2877	3.45	252.108627	1.72E+05	na	2	beam-type collision-induced dissociation	60	2934	136.061523	1.64E+04	116.047104	2.07	1, 124
1	4601	5.43	224.102966	5.57E+05	1	2	beam-type collision-induced dissociation	60	4647	108.055565	1.42E+04	116.047401	0.49	1
1	5897	6.94	294.119659	2.61E+05	1	2	beam-type collision-induced dissociation	60	5952	178.07222	5.41E+04	116.04744	0.82	1, 124
1	6183	7.28	340.150024	7.85E+04	na	2	beam-type collision-induced dissociation	60	6242	224.102753	3.10E+04	116.047272	0.62	1, 124
1	6435	7.57	252.109085	1.04E+05	1	2	beam-type collision-induced dissociation	60	6492	136.061661	3.99E+04	116.047424	0.69	1, 115, 124
1	6794	7.98	228.097778	2.52E+05	na	2	beam-type collision-induced dissociation	60	6847	112.050697	1.78E+04	116.047081	2.27	1, 85
1	7082	8.3	242.113373	1.16E+04	na	2	beam-type collision-induced dissociation	60	7123	126.066032	1.40E+04	116.04734	0.03	1
1	7082	8.31	280.090302	2.50E+05	1	2	beam-type collision-induced dissociation	60	7133	164.043304	7.81E+04	116.046997	2.99	1
1	7082	8.32	290.134613	6.87E+04	na	2	beam-type collision-induced dissociation	60	7143	174.087036	1.72E+04	116.047577	2.01	1, 124, 126
1	7405	8.67	258.108398	8.50E+06	1	2	beam-type collision-induced dissociation	60	7442	142.060913	6.54E+04	116.047485	1.22	1, 124
1	8478	9.93	252.109009	1.86E+05	1	2	beam-type collision-induced dissociation	60	8526	136.061813	4.56E+04	116.047195	1.28	1, 116, 124
1	8478	9.94	229.081787	4.84E+04	na	2	beam-type collision-induced dissociation	60	8537	113.034416	1.74E+04	116.047371	0.23	1, 90
1	8622	10.09	259.092316	6.78E+05	1	2	beam-type collision-induced dissociation	60	8668	143.04483	1.60E+04	116.047485	1.22	1, 85, 101, 124, 125
1	8622	10.1	281.07431	6.80E+05	1	2	beam-type collision-induced dissociation	60	8670	165.027008	3.36E+04	116.047302	0.36	1, 124
1	9269	10.84	284.09906	5.86E+04	na	2	beam-type collision-induced dissociation	60	9310	168.051743	1.33E+04	116.047318	0.23	1, 124, 125
1	9269	10.86	294.06958	6.06E+04	na	2	beam-type collision-induced dissociation	60	9334	178.022522	1.54E+04	116.047058	2.46	1

The output results were automatically classified based on the **tag [1]**.

Ions classified with Tag [1] are accompanied by annotations of **all matching tags**.

An example of output results from feature annotation and classification (Format-2)

When using a tag with **category 3 only** (see Appendix A4)

- The output results can be viewed in excel

Information of detected MS^1 peak pair (MS^1-MS$^{1'}$) with specific delta mass

Information of detected features

Classification by tag	MS Level	MS1 Scan Number	RT (min)	Precursor Ion m/z	Precursor Ion Intensity	Precursor Ion' m/z	Precursor Ion' Intensity	Delta Mass (Da)	Mass Error (ppm)	Artificial Adducts	Tags matched
124	1	2269	2.68	268.103972	3.69E+05	152.056664	7.74E+04	116.047308	0.31317	na	2, 83, 124, 125
124	1	2301	2.72	282.119527	2.73E+06	166.072272	3.24E+05	116.047255	0.768416	na	2, 12, 83, 124, 125, 126
124	1	2337	2.76	282.119566	1.61E+07	166.072322	2.26E+05	116.047244	0.862321	M+H	2, 124, 125, 126
124	1	2337	2.76	252.109154	5.18E+04	136.061833	9.45E+05	116.047322	0.193297	M+H	2, 124, 125, 126
124	1	2445	2.88	282.119651	6.97E+06	166.072299	8.55E+04	116.047352	0.072958	na	2, 12, 83, 85, 124, 125, 126
124	1	2481	2.92	282.119589	4.68E+06	166.072272	6.32E+04	116.047316	0.237533	na	2, 124, 125, 126
124	1	2517	2.97	252.109028	3.36E+04	136.061869	6.83E+05	116.047159	1.596199	na	1, 124
124	1	2553	3.01	268.104079	7.91E+04	152.056488	1.43E+04	116.047591	2.126877	na	2, 83, 124, 125
124	1	2589	3.05	282.119682	1.90E+06	166.072339	6.81E+04	116.047343	0.01156	na	2, 12, 83, 124, 125, 126
124	1	2697	3.17	268.104118	7.97E+04	152.056628	1.87E+04	116.04749	1.256413	M+H	2, 83, 124, 125
124	1	2697	3.17	252.108951	3.10E+04	136.061897	5.84E+05	116.047054	2.501978	M+H	2, 83, 124, 125
124	1	2733	3.21	282.11972	8.10E+05	166.072384	4.49E+04	116.047336	0.073181	na	2, 12, 124, 125, 126
124	1	2841	3.34	268.104094	1.05E+05	152.056656	2.03E+04	116.047438	0.812335	na	2, 124, 125
124	1	2877	3.38	282.119548	6.35E+05	166.072311	4.72E+04	116.047237	0.921835	na	2, 12, 124, 125, 126
124	1	2877	3.38	252.108668	3.75E+04	136.06181	5.91E+05	116.046857	4.193028	na	2, 12, 124, 125, 126
124	1	2913	3.42	282.119506	6.63E+05	166.072277	6.56E+04	116.047229	0.987507	na	2, 124, 125, 126
124	1	2985	3.5	268.104049	8.00E+04	152.056569	1.14E+04	116.047481	1.179333	na	2, 124, 125
124	1	3021	3.55	282.119591	3.83E+05	166.072323	4.89E+04	116.047269	0.648275	na	2, 124, 125, 126

An example of output results from feature annotation and classification (Format-3)

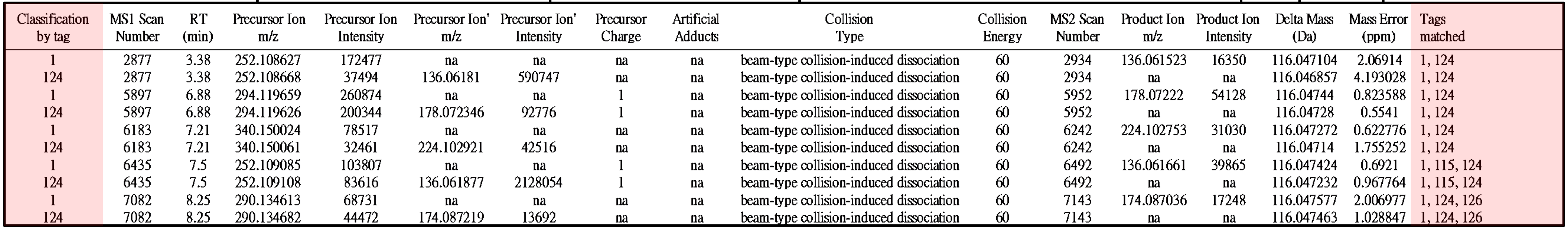
- The output results can be viewed in excel

When using a tag with **categories 1 and 3, or 2 and 3** (see Appendix A4)

Information of detected MS^1 peak pair (MS^1-MS$^{1'}$) with specific delta mass

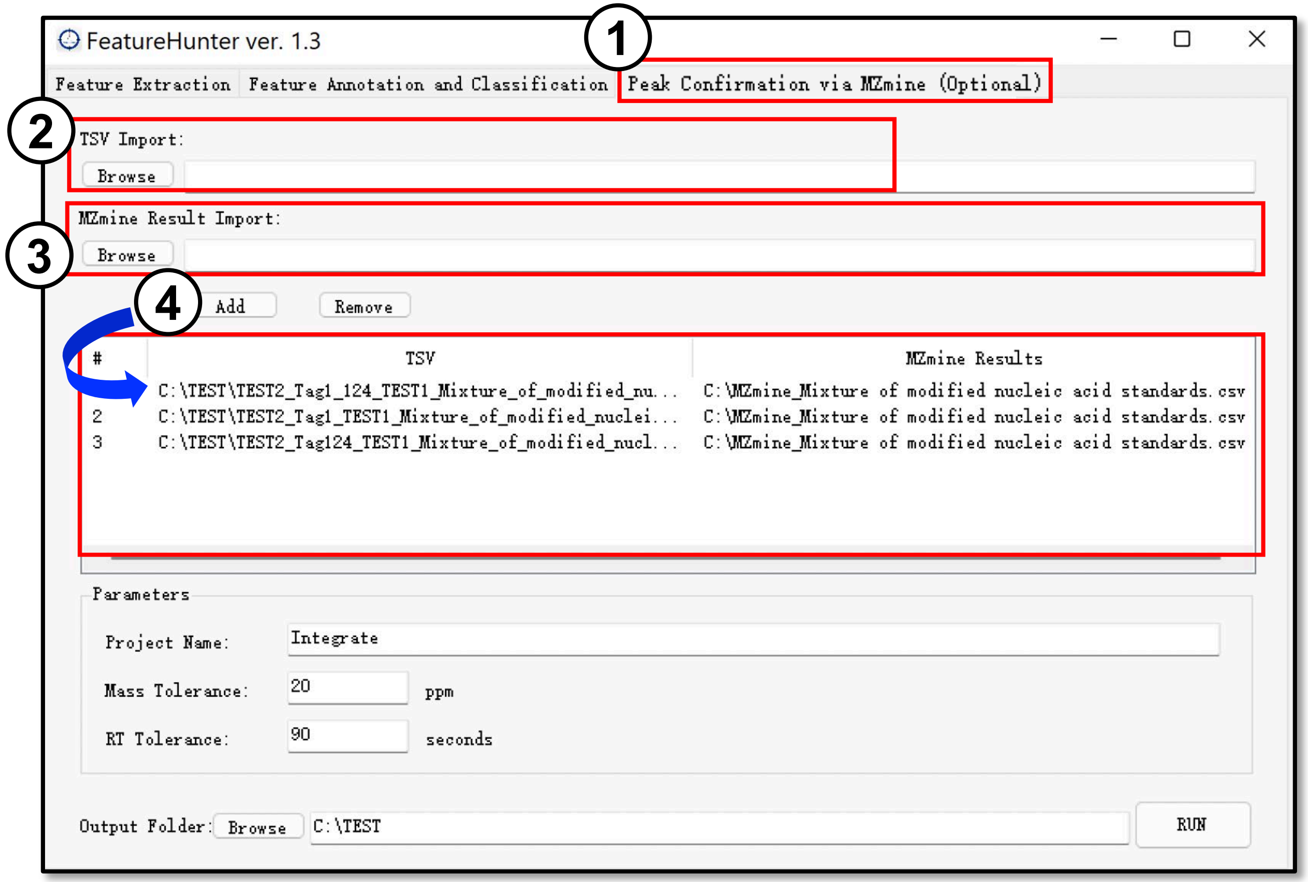
Information of detected MS^2 ions

Information of detected features



Classification by tag	MS1 Scan Number	RT (min)	Precursor Ion m/z	Precursor Ion Intensity	Precursor Ion' m/z	Precursor Ion' Intensity	Precursor Charge	Artificial Adducts	Collision Type	Collision Energy	MS2 Scan Number	Product Ion m/z	Product Ion Intensity	Delta Mass (Da)	Mass Error (ppm)	Tags matched
1	2877	3.38	252.108627	172477	na	na	na	na	beam-type collision-induced dissociation	60	2934	136.061523	16350	116.047104	2.06914	1, 124
124	2877	3.38	252.108668	37494	136.06181	590747	na	na	beam-type collision-induced dissociation	60	2934	na	na	116.046857	4.193028	1, 124
1	5897	6.88	294.119659	260874	na	na	1	na	beam-type collision-induced dissociation	60	5952	178.07222	54128	116.04744	0.823588	1, 124
124	5897	6.88	294.119626	200344	178.072346	92776	1	na	beam-type collision-induced dissociation	60	5952	na	na	116.04728	0.5541	1, 124
1	6183	7.21	340.150024	78517	na	na	na	na	beam-type collision-induced dissociation	60	6242	224.102753	31030	116.047272	0.622776	1, 124
124	6183	7.21	340.150061	32461	224.102921	42516	na	na	beam-type collision-induced dissociation	60	6242	na	na	116.04714	1.755252	1, 124
1	6435	7.5	252.109085	103807	na	na	1	na	beam-type collision-induced dissociation	60	6492	136.061661	39865	116.047424	0.6921	1, 115, 124
124	6435	7.5	252.109108	83616	136.061877	2128054	1	na	beam-type collision-induced dissociation	60	6492	na	na	116.047232	0.967764	1, 115, 124
1	7082	8.25	290.134613	68731	na	na	na	na	beam-type collision-induced dissociation	60	7143	174.087036	17248	116.047577	2.006977	1, 124, 126
124	7082	8.25	290.134682	44472	174.087219	13692	na	na	beam-type collision-induced dissociation	60	7143	na	na	116.047463	1.028847	1, 124, 126

3-4. Peak Confirmation via MZmine (optional function) [Step-4; optional]



#	TSV	MZmine Results
	C:\TEST\TEST2_Tag1_124_TEST1_Mixture_of_modified_nu...	C:\MZmine_Mixture of modified nucleic acid standards.csv
2	C:\TEST\TEST2_Tag1_TEST1_Mixture_of_modified_nuclei...	C:\MZmine_Mixture of modified nucleic acid standards.csv
3	C:\TEST\TEST2_Tag124_TEST1_Mixture_of_modified_nucl...	C:\MZmine_Mixture of modified nucleic acid standards.csv

Project Name: Integrate

Mass Tolerance: 20 ppm

RT Tolerance: 90 seconds

Output Folder: C:\TEST

Fig-12. Interface of “Peak Confirmation via MZmine” in *FeatureHunter*

① Select the “(optional) Peak Confirmation via MZmine”.

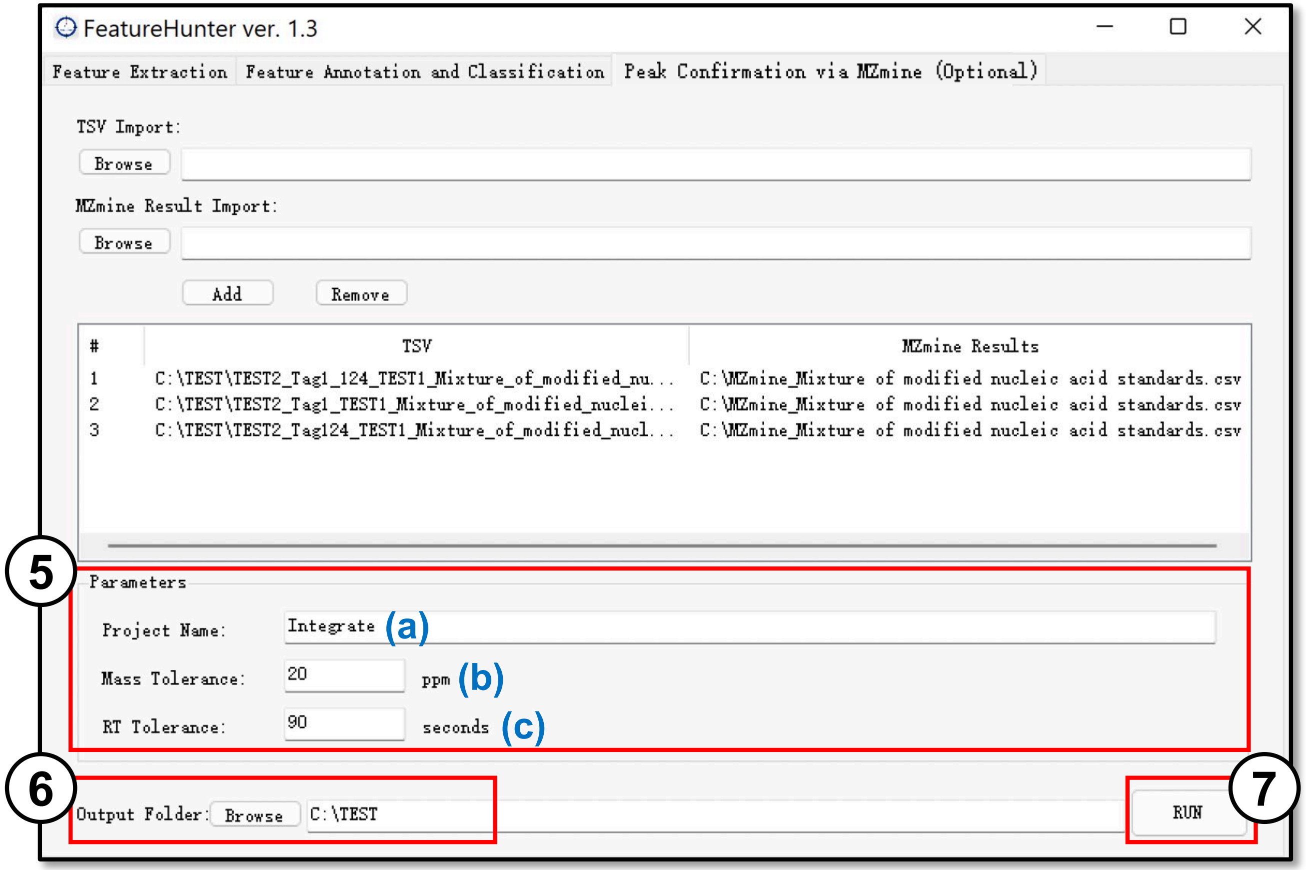
② Click “Browse” to import the .tsv file that was generated from Feature Annotation and Classification (Function-2; step-3).

③ Click “Browse” to import the .csv file that was outputted from MZmine[1].

④ Click “Add” to submit the data file imported from *FeatureHunter* (.tsv) and MZmine (.csv) for data integration[2].

- The configuration of the required elements in MZmine's output file is described in **Appendix A6**.
- *FeatureHunter* enables integrating single, multiple results (from different tags), or aligned results (from different samples) with the MZmine file. (see **Appendix A7**)

3-4. Peak Confirmation via MZmine (optional function) [Step-4; optional]



#	TSV	MZmine Results
1	C:\TEST\TEST2_Tag1_124_TEST1_Mixture_of_modified_nu...	C:\MZmine_Mixture of modified nucleic acid standards.csv
2	C:\TEST\TEST2_Tag1_TEST1_Mixture_of_modified_nuclei...	C:\MZmine_Mixture of modified nucleic acid standards.csv
3	C:\TEST\TEST2_Tag124_TEST1_Mixture_of_modified_nucl...	C:\MZmine_Mixture of modified nucleic acid standards.csv

Project Name: Integrate
Mass Tolerance: 20 ppm
RT Tolerance: 90 seconds
Output Folder: C:\TEST

Fig-12. Interface of “Peak Confirmation via MZmine” in *FeatureHunter*

⑤ **Set the parameters:**

(a) Specify a "Project Name" which will be automatically added as a prefix to the output file name. For example: **Integrate**_file name

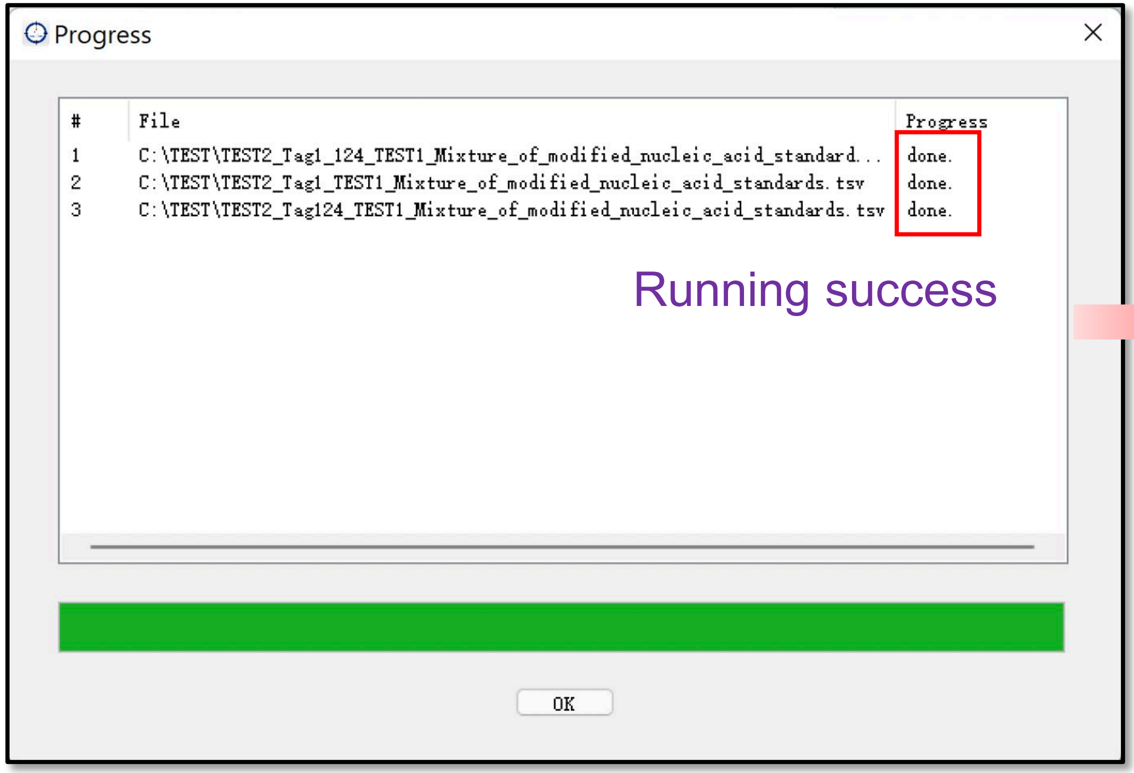
(b) Set the “Mass Tolerance” with ppm for intersecting the precursor ion m/z between *FeatureHunter’s* and MZmine’s output lists.

(c) Set the “RT Tolerance” with seconds for intersecting the precursor ion RT between *FeatureHunter’s* and MZmine’s output lists.

⑥ Specify the output path and folder. (The output files will be in .csv format.)

⑦ Click "RUN" to start execution.

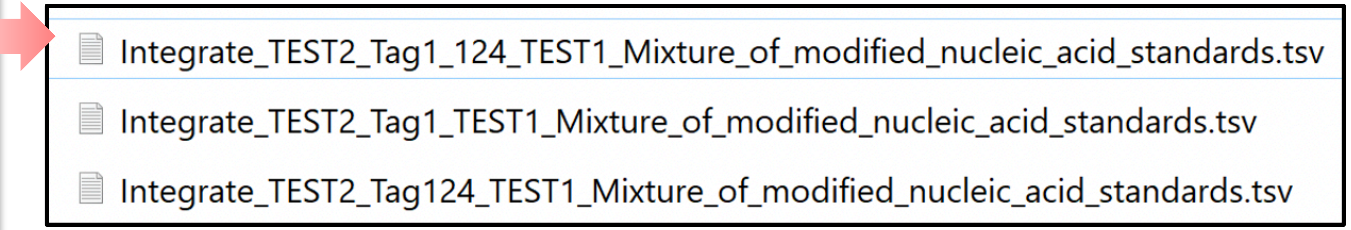
Finished



#	File	Progress
1	C:\TEST\TEST2_Tag1_124_TEST1_Mixture_of_modified_nucleic_acid_standard...	done.
2	C:\TEST\TEST2_Tag1_TEST1_Mixture_of_modified_nucleic_acid_standards.tsv	done.
3	C:\TEST\TEST2_Tag124_TEST1_Mixture_of_modified_nucleic_acid_standards.tsv	done.

Running success

Output success



Integrate_TEST2_Tag1_124_TEST1_Mixture_of_modified_nucleic_acid_standards.tsv
Integrate_TEST2_Tag1_TEST1_Mixture_of_modified_nucleic_acid_standards.tsv
Integrate_TEST2_Tag124_TEST1_Mixture_of_modified_nucleic_acid_standards.tsv

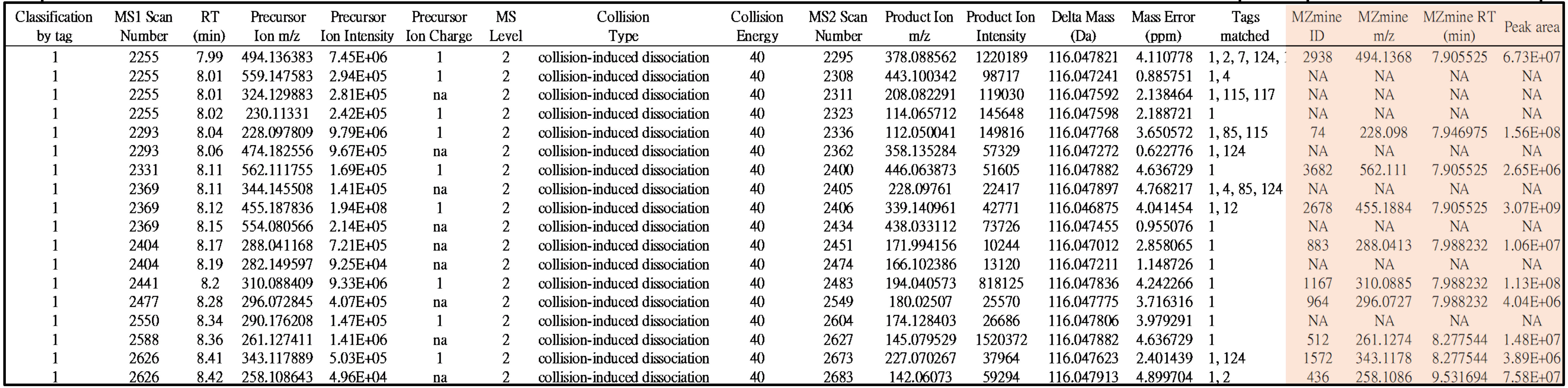
Fig-13. The integrated results between *FeatureHunter* and MZmine are output in the specified output folder.

An example of output results from data integration

- <u>The output results can be viewed in excel</u>

Information from ***FeatureHunter***

Information from ***MZmine***

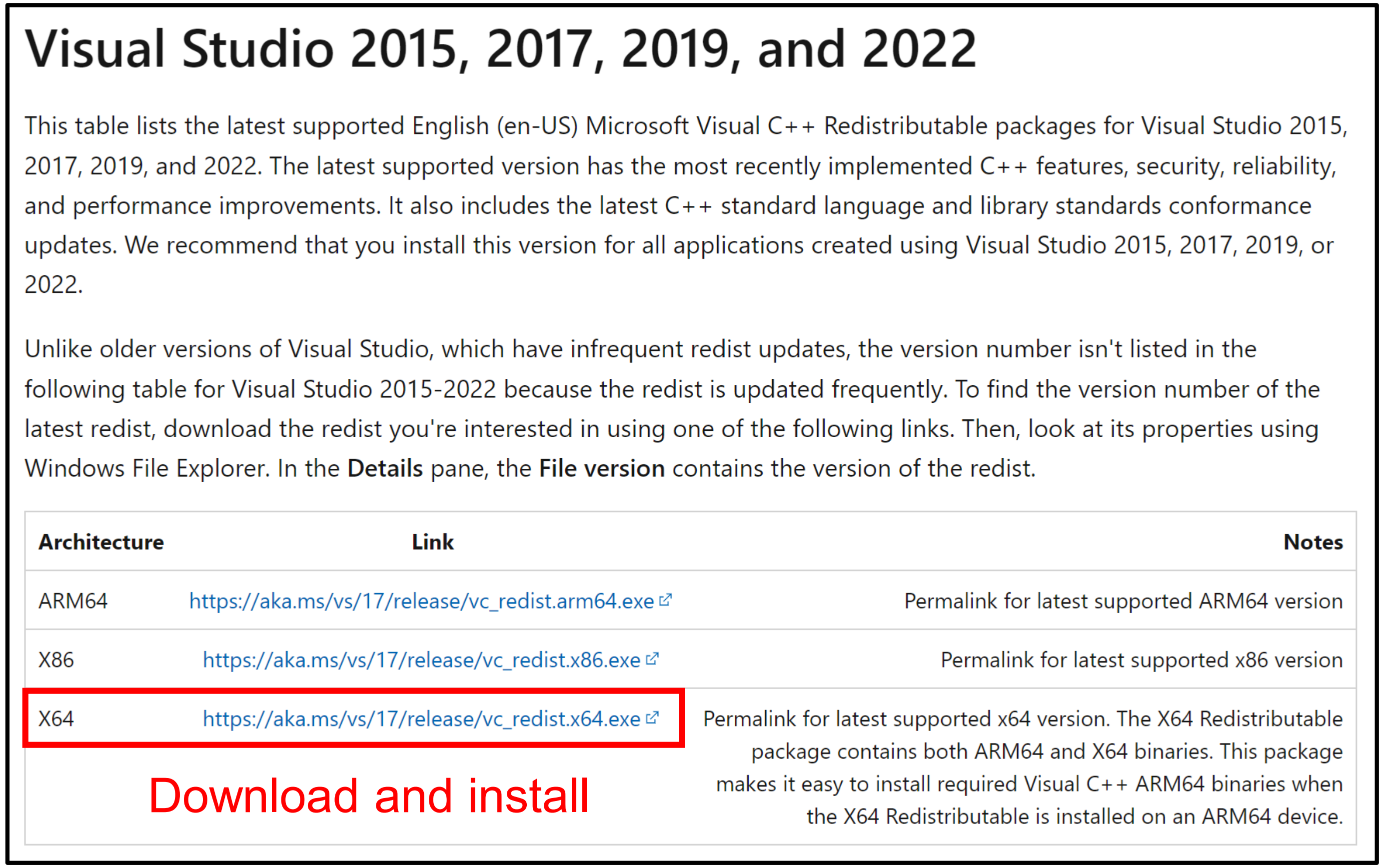


Classification by tag	MS1 Scan Number	RT (min)	Precursor Ion m/z	Precursor Ion Intensity	Precursor Ion Charge	MS Level	Collision Type	Collision Energy	MS2 Scan Number	Product Ion m/z	Product Ion Intensity	Delta Mass (Da)	Mass Error (ppm)	Tags matched	MZmine ID	MZmine m/z	MZmine RT (min)	Peak area
1	2255	7.99	494.136383	7.45E+06	1	2	collision-induced dissociation	40	2295	378.088562	1220189	116.047821	4.110778	1, 2, 7, 124,	2938	494.1368	7.905525	6.73E+07
1	2255	8.01	559.147583	2.94E+05	1	2	collision-induced dissociation	40	2308	443.100342	98717	116.047241	0.885751	1, 4	NA	NA	NA	NA
1	2255	8.01	324.129883	2.81E+05	na	2	collision-induced dissociation	40	2311	208.082291	119030	116.047592	2.138464	1, 115, 117	NA	NA	NA	NA
1	2255	8.02	230.11331	2.42E+05	1	2	collision-induced dissociation	40	2323	114.065712	145648	116.047598	2.188721	1	NA	NA	NA	NA
1	2293	8.04	228.097809	9.79E+06	1	2	collision-induced dissociation	40	2336	112.050041	149816	116.047768	3.650572	1, 85, 115	74	228.098	7.946975	1.56E+08
1	2293	8.06	474.182556	9.67E+05	na	2	collision-induced dissociation	40	2362	358.135284	57329	116.047272	0.622776	1, 124	NA	NA	NA	NA
1	2331	8.11	562.111755	1.69E+05	1	2	collision-induced dissociation	40	2400	446.063873	51605	116.047882	4.636729	1	3682	562.111	7.905525	2.65E+06
1	2369	8.11	344.145508	1.41E+05	na	2	collision-induced dissociation	40	2405	228.09761	22417	116.047897	4.768217	1, 4, 85, 124	NA	NA	NA	NA
1	2369	8.12	455.187836	1.94E+08	1	2	collision-induced dissociation	40	2406	339.140961	42771	116.046875	4.041454	1, 12	2678	455.1884	7.905525	3.07E+09
1	2369	8.15	554.080566	2.14E+05	na	2	collision-induced dissociation	40	2434	438.033112	73726	116.047455	0.955076	1	NA	NA	NA	NA
1	2404	8.17	288.041168	7.21E+05	na	2	collision-induced dissociation	40	2451	171.994156	10244	116.047012	2.858065	1	883	288.0413	7.988232	1.06E+07
1	2404	8.19	282.149597	9.25E+04	na	2	collision-induced dissociation	40	2474	166.102386	13120	116.047211	1.148726	1	NA	NA	NA	NA
1	2441	8.2	310.088409	9.33E+06	1	2	collision-induced dissociation	40	2483	194.040573	818125	116.047836	4.242266	1	1167	310.0885	7.988232	1.13E+08
1	2477	8.28	296.072845	4.07E+05	na	2	collision-induced dissociation	40	2549	180.02507	25570	116.047775	3.716316	1	964	296.0727	7.988232	4.04E+06
1	2550	8.34	290.176208	1.47E+05	1	2	collision-induced dissociation	40	2604	174.128403	26686	116.047806	3.979291	1	NA	NA	NA	NA
1	2588	8.36	261.127411	1.41E+06	na	2	collision-induced dissociation	40	2627	145.079529	1520372	116.047882	4.636729	1	512	261.1274	8.277544	1.48E+07
1	2626	8.41	343.117889	5.03E+05	1	2	collision-induced dissociation	40	2673	227.070267	37964	116.047623	2.401439	1, 124	1572	343.1178	8.277544	3.89E+06
1	2626	8.42	258.108643	4.96E+04	na	2	collision-induced dissociation	40	2683	142.06073	59294	116.047913	4.899704	1, 2	436	258.1086	9.531694	7.58E+07

⇩

<u>**NA**</u> indicates that the ions were not detected in MZmine. It is recommended to exclude these ions from the final results due to their low peak quality and confidence.

4. Appendix-A1



Visual Studio 2015, 2017, 2019, and 2022

This table lists the latest supported English (en-US) Microsoft Visual C++ Redistributable packages for Visual Studio 2015, 2017, 2019, and 2022. The latest supported version has the most recently implemented C++ features, security, reliability, and performance improvements. It also includes the latest C++ standard language and library standards conformance updates. We recommend that you install this version for all applications created using Visual Studio 2015, 2017, 2019, or 2022.

Unlike older versions of Visual Studio, which have infrequent redist updates, the version number isn't listed in the following table for Visual Studio 2015-2022 because the redist is updated frequently. To find the version number of the latest redist, download the redist you're interested in using one of the following links. Then, look at its properties using Windows File Explorer. In the **Details** pane, the **File version** contains the version of the redist.

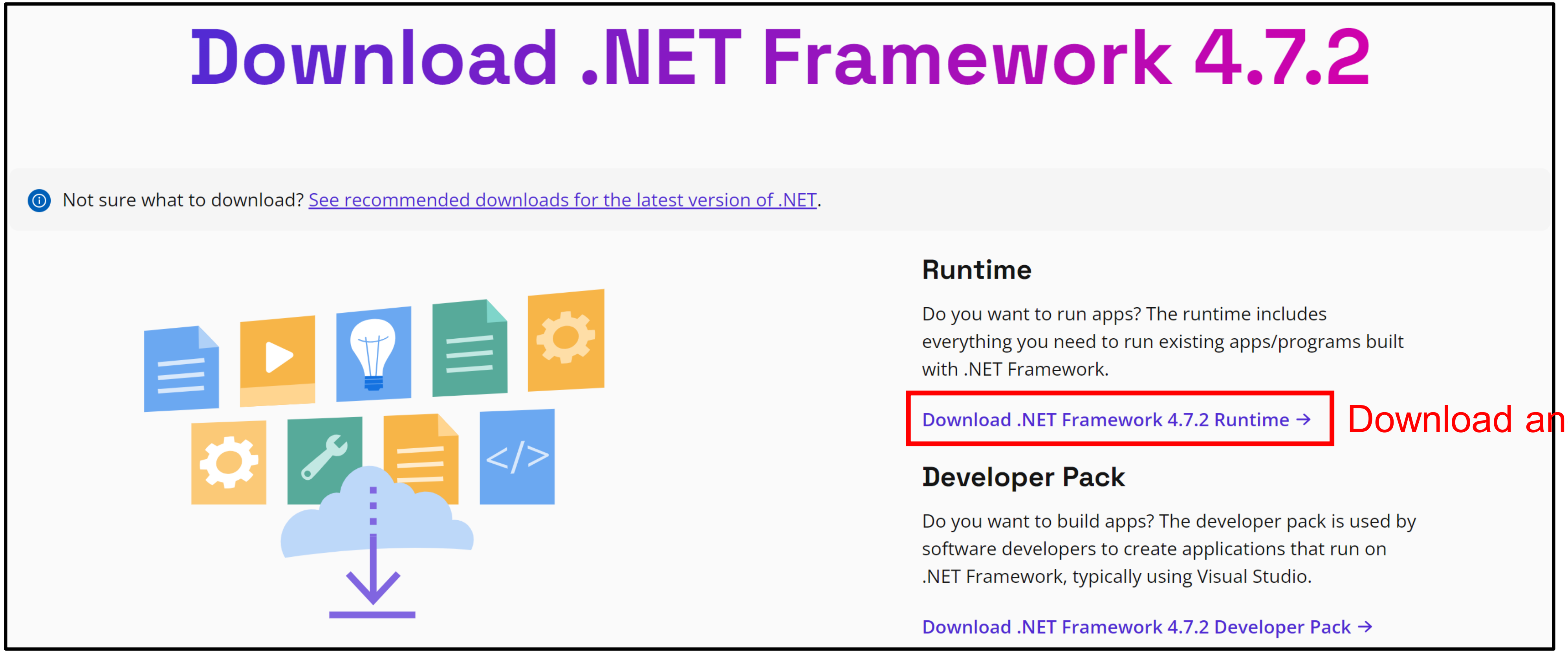
Architecture	Link	Notes
ARM64	https://aka.ms/vs/17/release/vc_redist.arm64.exe	Permalink for latest supported ARM64 version
X86	https://aka.ms/vs/17/release/vc_redist.x86.exe	Permalink for latest supported x86 version
X64	https://aka.ms/vs/17/release/vc_redist.x64.exe	Permalink for latest supported x64 version. The X64 Redistributable package contains both ARM64 and X64 binaries. This package makes it easy to install required Visual C++ ARM64 binaries when the X64 Redistributable is installed on an ARM64 device.

Download and install

Fig-A1. The page for downloading Visual C++ redistributable package

Visual C++ redistributable package can be downloaded from the official website of Microsoft:
https://learn.microsoft.com/en-us/cpp/windows/latest-supported-vc-redist?view=msvc-170

4. Appendix-A2



Download .NET Framework 4.7.2

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Download and install

Developer Pack

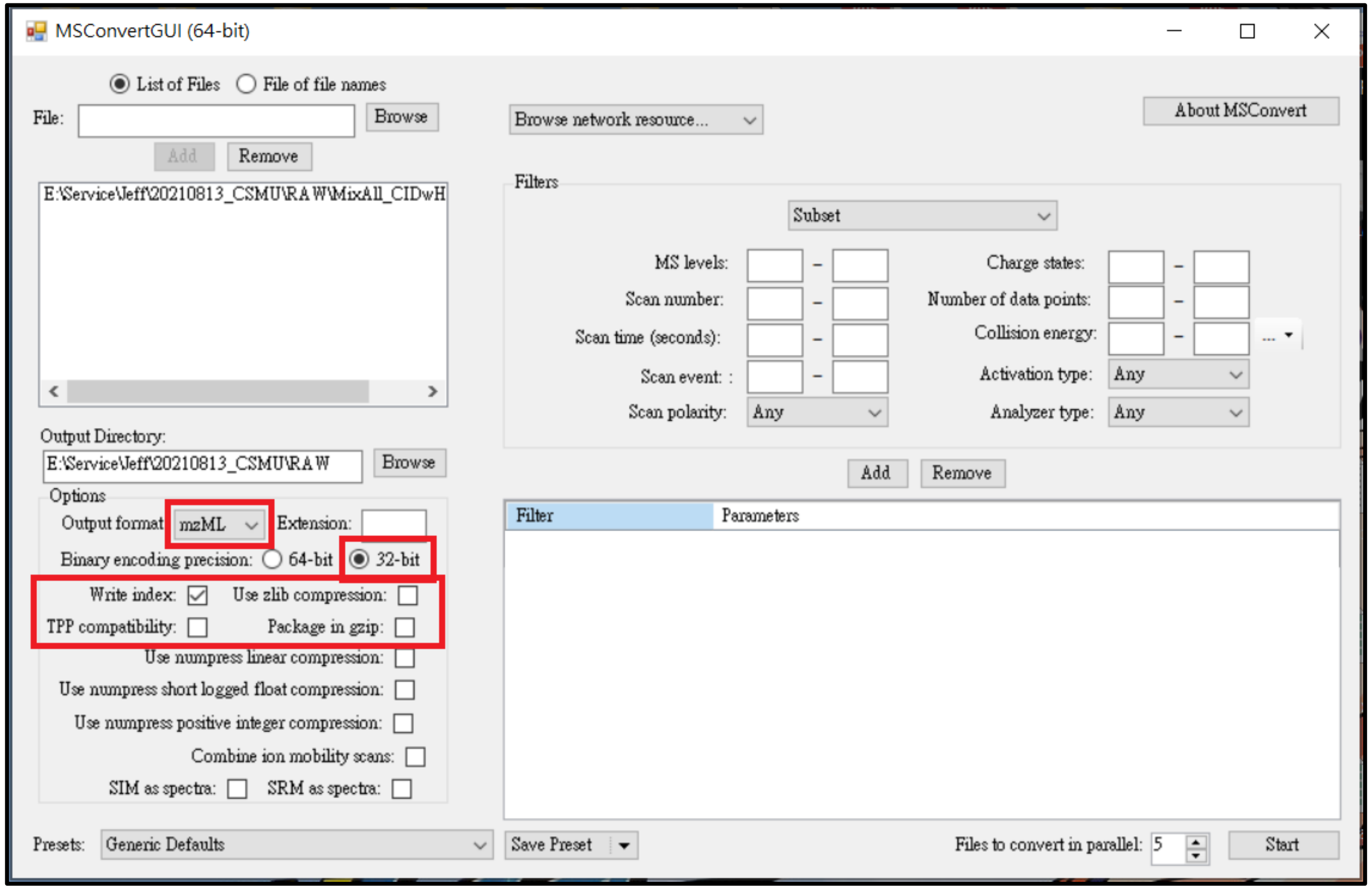
Do you want to build apps? The developer pack is used by software developers to create applications that run on .NET Framework, typically using Visual Studio.

Download .NET Framework 4.7.2 Developer Pack →

Fig-A2. The page for downloading .NET Framework

.NET Framwork can be downloaded from the official website of Microsoft:
https://dotnet.microsoft.com/en-us/download/dotnet-framework/net472

4. Appendix-A3



● List of Files ○ File of file names

File:

E:\Service\Jeff\20210813_CSMU\RAW\MixAll_CIDwH

Output Directory: E:\Service\Jeff\20210813_CSMU\RAW

Options

Output format: mzML

Extension:

Binary encoding precision: ○ 64-bit ● 32-bit

Write index: ☑ Use zlib compression: ☐

TPP compatibility: ☐ Package in gzip: ☐

Use numpress linear compression: ☐

Use numpress short logged float compression: ☐

Use numpress positive integer compression: ☐

Combine ion mobility scans: ☐

SIM as spectra: ☐ SRM as spectra: ☐

Subset

MS levels: –

Scan number: –

Scan time (seconds): –

Scan event: : –

Scan polarity: Any

Charge states: –

Number of data points: –

Collision energy: –

Activation type: Any

Analyzer type: Any

Presets: Generic Defaults

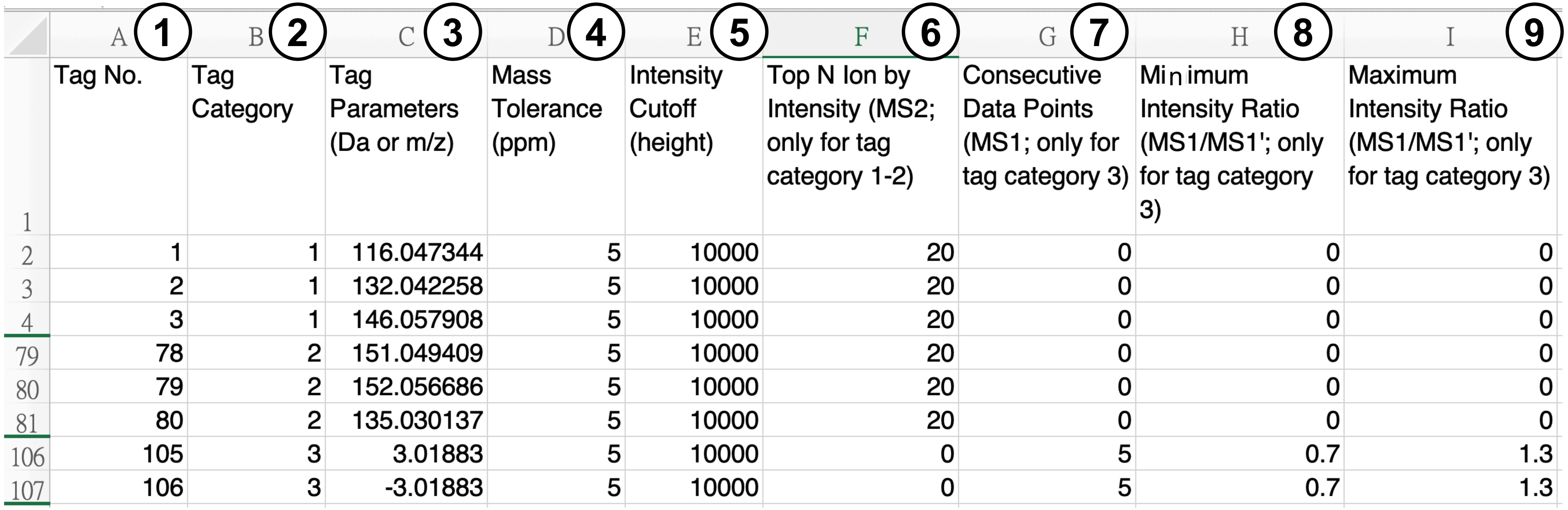
Files to convert in parallel: 5

Fig-A3. Recommended setting parameter for converting data from vendor’s format to mzML

MSConvert can be downloaded from the website:

https://proteowizard.sourceforge.io/download.html

4. Appendix-A4



	A ①	B ②	C ③	D ④	E ⑤	F ⑥	G ⑦	H ⑧	I ⑨
1	Tag No.	Tag Category	Tag Parameters (Da or m/z)	Mass Tolerance (ppm)	Intensity Cutoff (height)	Top N Ion by Intensity (MS2; only for tag category 1-2)	Consecutive Data Points (MS1; only for tag category 3)	Minimum Intensity Ratio (MS1/MS1'; only for tag category 3)	Maximum Intensity Ratio (MS1/MS1'; only for tag category 3)
2	1	1	116.047344	5	10000	20	0	0	0
3	2	1	132.042258	5	10000	20	0	0	0
4	3	1	146.057908	5	10000	20	0	0	0
79	78	2	151.049409	5	10000	20	0	0	0
80	79	2	152.056686	5	10000	20	0	0	0
81	80	2	135.030137	5	10000	20	0	0	0
106	105	3	3.01883	5	10000	0	5	0.7	1.3
107	106	3	-3.01883	5	10000	0	5	0.7	1.3

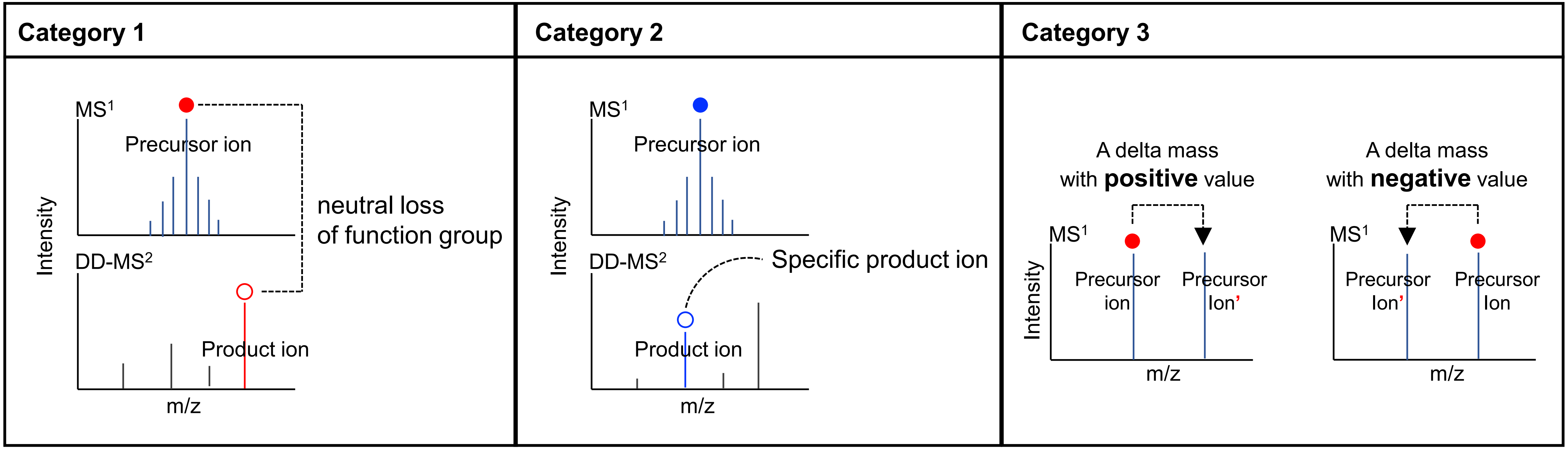
Fig-A4. The management of Feature List.csv

1. Tag No.: Tag No. is utilized to characterize the tag parameters in *FeatureHunter*. Each tag represents a specific feature or characteristic that can be assigned to annotated data for classification purpose. The default Tag No. ranges from 1 to 132. Users have the flexibility to manage the feature list by adding, editing, or removing tags as needed.

2. Tag Category.: Tag category is utilized to classify tags into three types.

These categories include:

- Tag category 1: neutral loss of a particular functional group.
- Tag category 2: specific diagnostic product ion m/z in MS^2.
- Tag category 3: a fixed delta mass in MS^1.



3. Tag Parameters: Tag parameters are utilized to extract the diagnostic features of the analytes of interest obtained from MS^1 or MS^2 raw data.

4. Mass Tolerance: Mass tolerance is utilized for tag parameters with a default value of 5 ppm. Users can adjust this value to meet their data quality requirements.

5. Intensity Cutoff (height): Intensity cutoff is utilized to filter features (such as product ions or peak pairs) based on their peak height, retaining only those above the cutoff value. The default value is 10000. Users can adjust this value to meet their data quality requirements.

6. Top N ion by intensity: The Top N ions filter is utilized to retain product ions with intensity rankings within the top N in the MS^2 spectrum. This function is applicable only to categories 1 and 2. The default value is 20. Users can adjust this value to meet their data quality requirements. The function can be disable by setting the value to zero.

7. Consecutive Data Points: Consecutive data points are utilized to filter specific MS^1 peak-pairs with a fixed delta mass across consecutive MS^1 spectra (data points). This function is applicable only to category 3. The default value is 5. Users can adjust this value to meet their data quality requirements. The function can be disable by setting the value to zero.

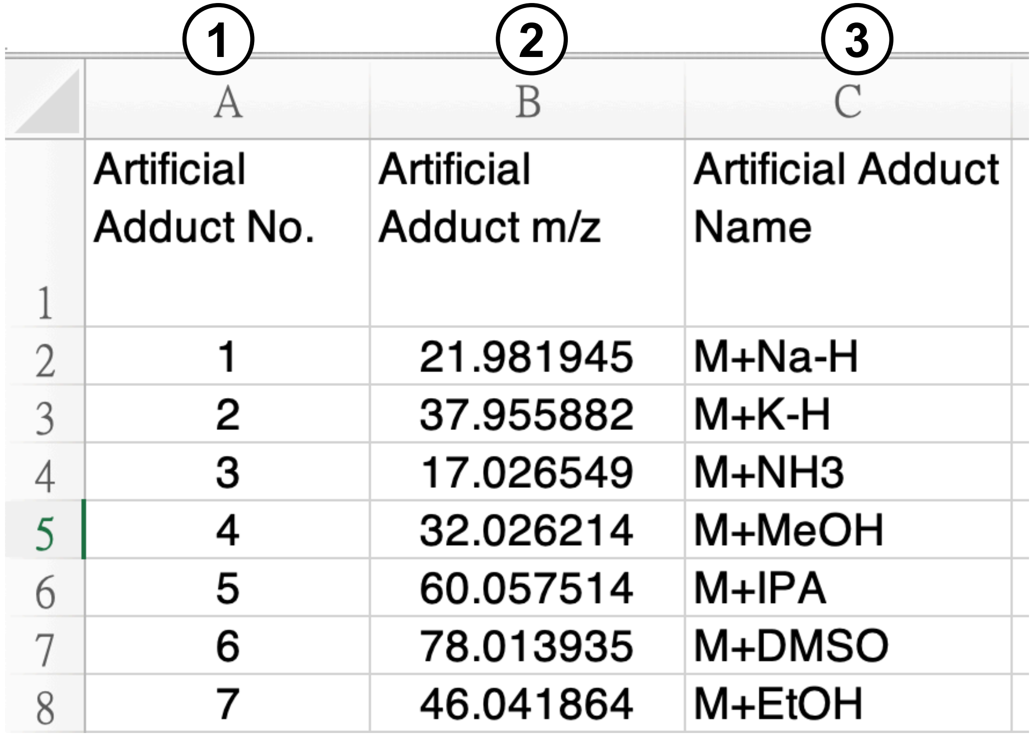
8. Minimum Intensity Ratio: The minimum intensity ratio is utilized to filter specific MS^1 peak-pairs based on a specified intensity ratio. The default value is 0.7. Users can adjust this value to meet their data quality requirements. The function can be disable by setting the value to zero.

- Intensity ratio of peak-pairs: $\frac{\text{Precursor ion}}{\text{Precursor ion'}}$

9. Maximum Intensity Ratio : The maximum intensity ratio is utilized to filter specific MS^1 peak-pairs based on a specified intensity ratio. The default value is 1.3. Users can adjust this value to meet their data quality requirements. The function can be disable by setting the value to zero.

- Intensity ratio of peak-pairs: $\frac{\text{Precursor ion}}{\text{Precursor ion'}}$

4. Appendix-A5



Artificial Adduct No.	Artificial Adduct m/z	Artificial Adduct Name
1	21.981945	M+Na-H
2	37.955882	M+K-H
3	17.026549	M+NH3
4	32.026214	M+MeOH
5	60.057514	M+IPA
6	78.013935	M+DMSO
7	46.041864	M+EtOH

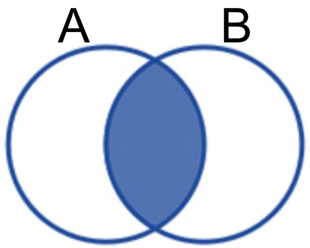
Fig-A5. The management of Artificial Adducts List

1. Artificial Adduct No.: The ID of each artificial adduct in the list.

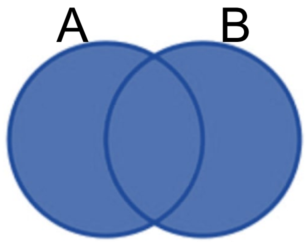
2. Artificial Adduct m/z: Artificial Adduct m/z is used to annotate the adduct ion of the precursor ion in the MS^1 spectrum. The default artificial adduct list comprises 13 common artificial adducts. Users have the flexibility to manage the feature list by adding, editing, or removing artificial adducts as needed.

3. Artificial Adduct Name: Artificial Adduct name is used for characterizing the identity of artificial adducts.

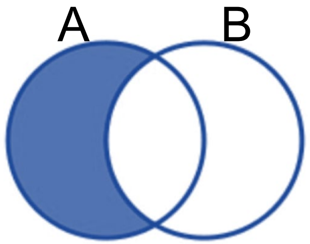
5. Appendix-A6



Intersection
[A∩B]



Union
[A∪B]



Difference
[A−B]

Example:

- **[1]:**

  Intersection: 1 Add

- **[1∩124]:**

  Intersection: 1,124 Add

- **[2∪3∪4]:**

  Union: 2,3,4 Add

- **[1∩124] − [2]:**

  Intersection: 1,124
  Difference: 2
  Add

- **[2∪3] − [4]:**

  Union: 2,3
  Difference: 4
  Add

- **[1] ∩ [2∪3] ∩ [7∪8]:**

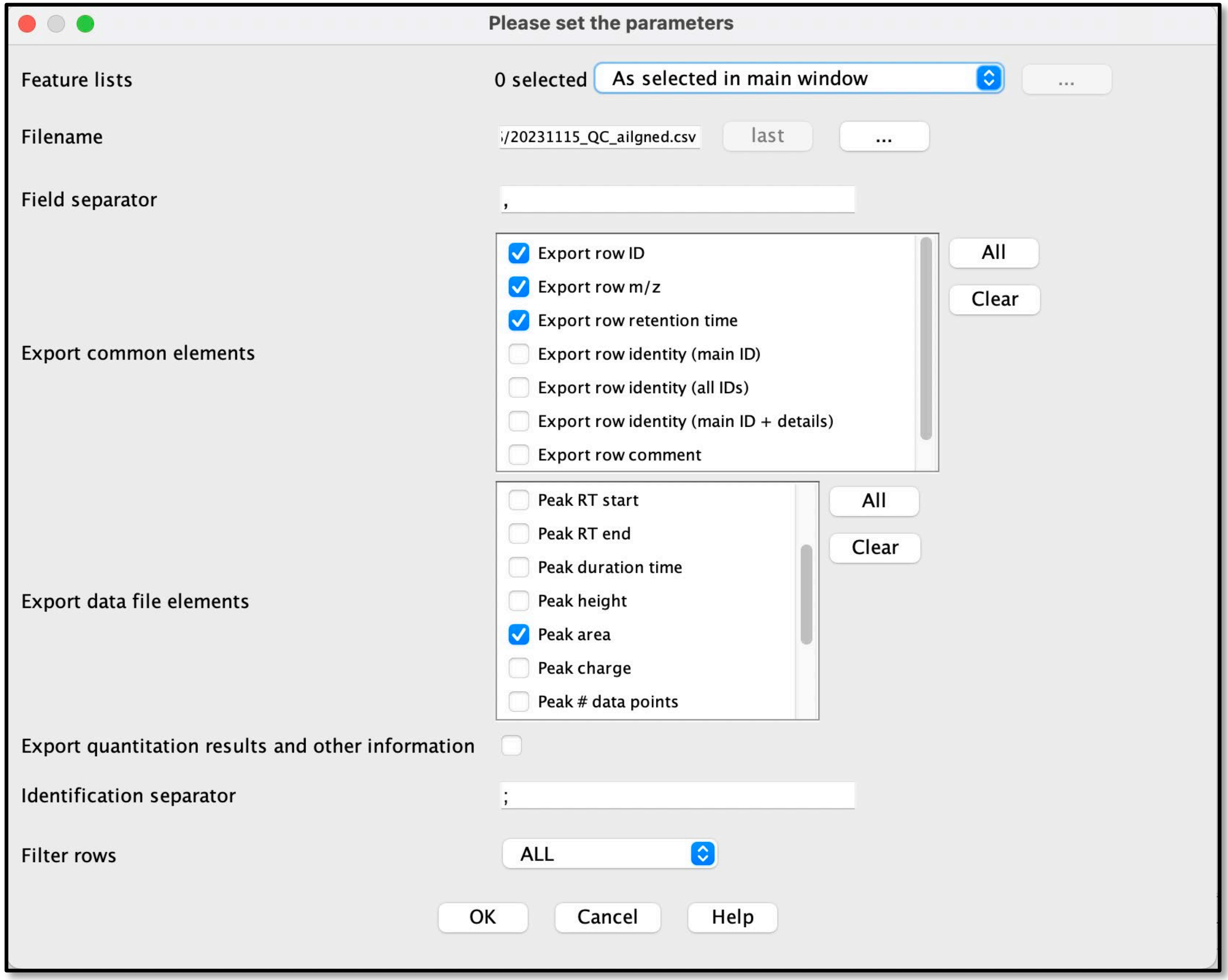
  Step-1 Intersection: 1
  Union: 2,3
  Add

  Step-2 Union: 7,8 Add

- **[42] ∩ [58] ∩ [78∪79∪80] − [115∪116]:**

  Intersection: 42,58
  Union: 78,79,80
  Difference: 115,116
  Add

5. Appendix-A7



Please set the parameters

Feature lists: 0 selected — As selected in main window

Filename: /20231115_QC_ailgned.csv

Field separator: ,

Export common elements:
- ☑ Export row ID
- ☑ Export row m/z
- ☑ Export row retention time
- ☐ Export row identity (main ID)
- ☐ Export row identity (all IDs)
- ☐ Export row identity (main ID + details)
- ☐ Export row comment

Export data file elements:
- ☐ Peak RT start
- ☐ Peak RT end
- ☐ Peak duration time
- ☐ Peak height
- ☑ Peak area
- ☐ Peak charge
- ☐ Peak # data points

Export quantitation results and other information ☐

Identification separator: ;

Filter rows: ALL

Fig-A6. The configuration of required elements for data output from MZmine

FeatureHunter has been verified to support data integration with file exported from both older (2.53) and latest versions (3.90) of MZmine.

The processed results, exported as a .CSV file from MZmine, should include the following elements:

(1) Row ID

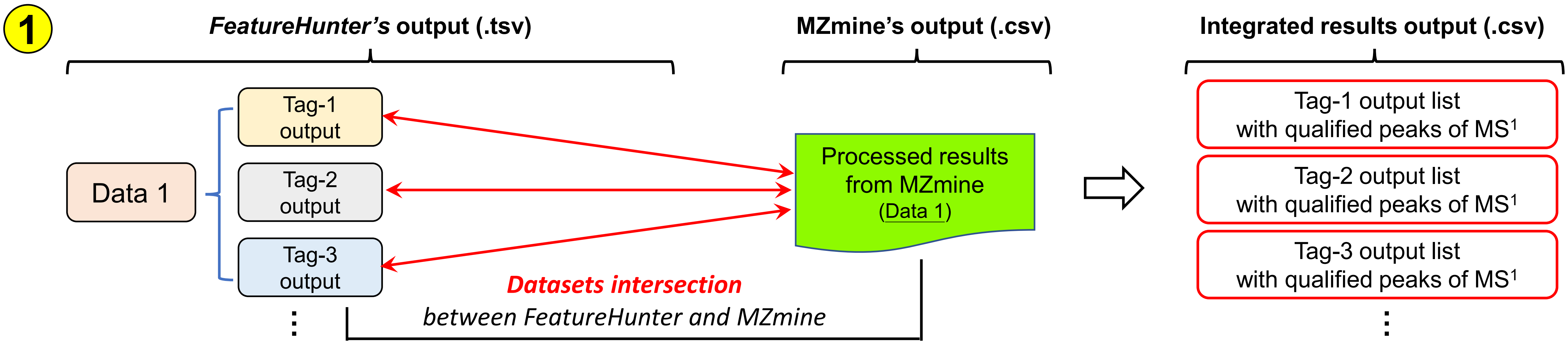
(2) Row m/z

(3) Row retention time

(4) Peak area

When exporting data from MZmine 3, select "Export CSV (legacy MZmine 2)".

5. Appendix-A8



①

***FeatureHunter's* output (.tsv)**

MZmine's output (.csv)

Integrated results output (.csv)

Data 1

Tag-1 output

Tag-2 output

Tag-3 output

⋮

Processed results from MZmine (Data 1)

Datasets intersection

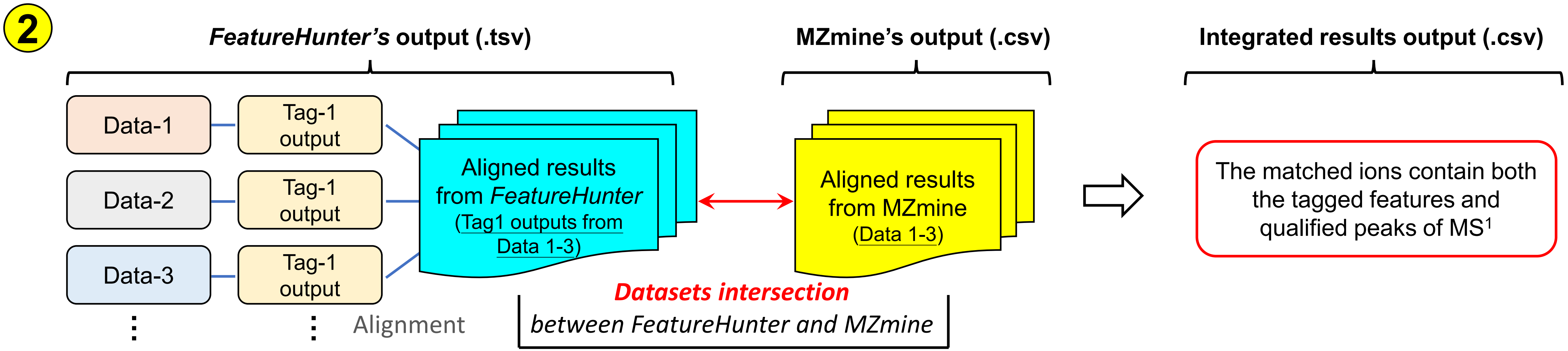
between FeatureHunter and MZmine

Tag-1 output list with qualified peaks of MS^1

Tag-2 output list with qualified peaks of MS^1

Tag-3 output list with qualified peaks of MS^1

⋮



②

***FeatureHunter's* output (.tsv)**

MZmine's output (.csv)

Integrated results output (.csv)

Data-1

Data-2

Data-3

⋮

Tag-1 output

Tag-1 output

Tag-1 output

⋮

Alignment

Aligned results from *FeatureHunter* (Tag1 outputs from Data 1-3)

Aligned results from MZmine (Data 1-3)

Datasets intersection

between FeatureHunter and MZmine

The matched ions contain both the tagged features and qualified peaks of MS^1

Report Issues

We welcome your feedback and comments, as they can greatly contribute to enhancing the development of the software.

> If you encounter any issues with *FeatureHunter*, please include the following information in your issue report:
>
> *(1) The version of FeatureHunter.*
>
> *(2) Screenshots of error messages or unexpected results.*
>
> *(3) Any additional information that could help us better understand the challenges or bugs you're encountering.*

Please leave your feedback and comments at the following link:

https://msomics.abrc.sinica.edu.tw/FeatureHunter/?page_id=271

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