MRM-Ion Pair Finder v2.0 user manual

MRM-Ion Pair Finder software is an independently developed and written program in our laboratory for large-scale selection of MRM transitions for pseudotargeted metabolomics. The Matlab (Version 7.14.739, R2012a,64-bit) is used. MRM-Ion Pair Finder v2.0 is improved on the basis of the original version, which simplifies the program and improves the running efficiency. Matlab language (R2018b, 64-bit) is used.

The whole process of running the software is as follows: (1) Establish folders containing parent ion information (parent ion information from the results of XCMS and CAMERA) and product ion information (product ion information from MGF files). (2) Set parameters, run MRM-Ion Pair Finder v2.0 software, automatically select ion pairs, output results.

The instructions for the installation and use of the software are shown below:

1 Download and extract MRM-Ion Pair Finder v2.0.zip.

2 Select the extracted MRM-Ion Pair Finder v2.0 software folder in Matlab, as shown in **Figure 1**.

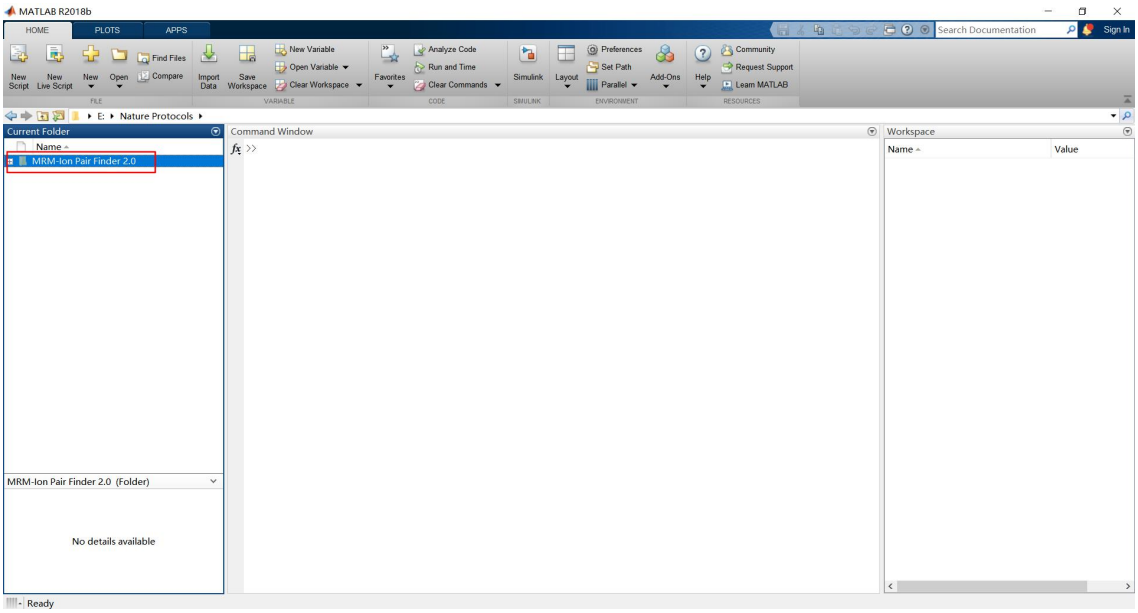

Figure 1 | Select the MRM-Ion Pair Finder software folder location.

3 Load data file path: Add to Path → Selected Folders and Subfolders

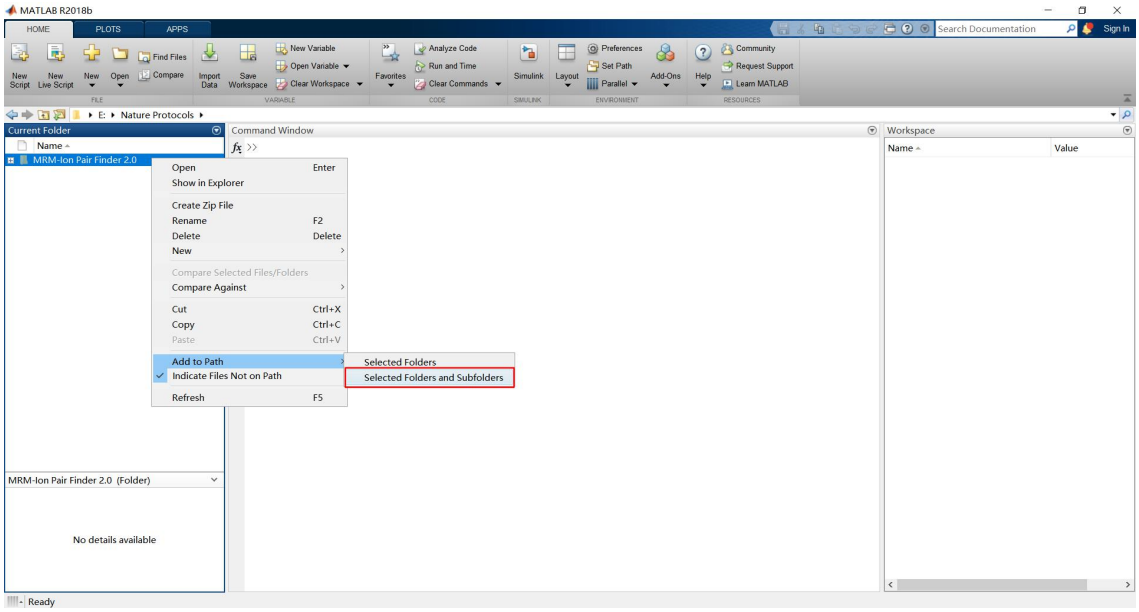

Figure 2 | Load data file path

4 Prepare MS1 file (csv) and MS2 files (mgf). MS1 format for MRM-Ion Pair Finder v2.0 is shown in **Figure 6**. PeakView (AB SCIEX, Version: 1.2.0.3) is used for transfer raw data (.wiff) to mgf file. MSConvert can also be used to get mgf files from different instruments raw file types. Peak peaking algorithm are set as continuous wavelet transform (cwt) in MSConvert. Other filters can make the data simpler and reduce the running time of MRM-Ion Pair Finder v2.0.

5 Open folder of MRM-Ion Pair Finder v2.0, Double-click "NEW_FINDER.fig" in the left column.

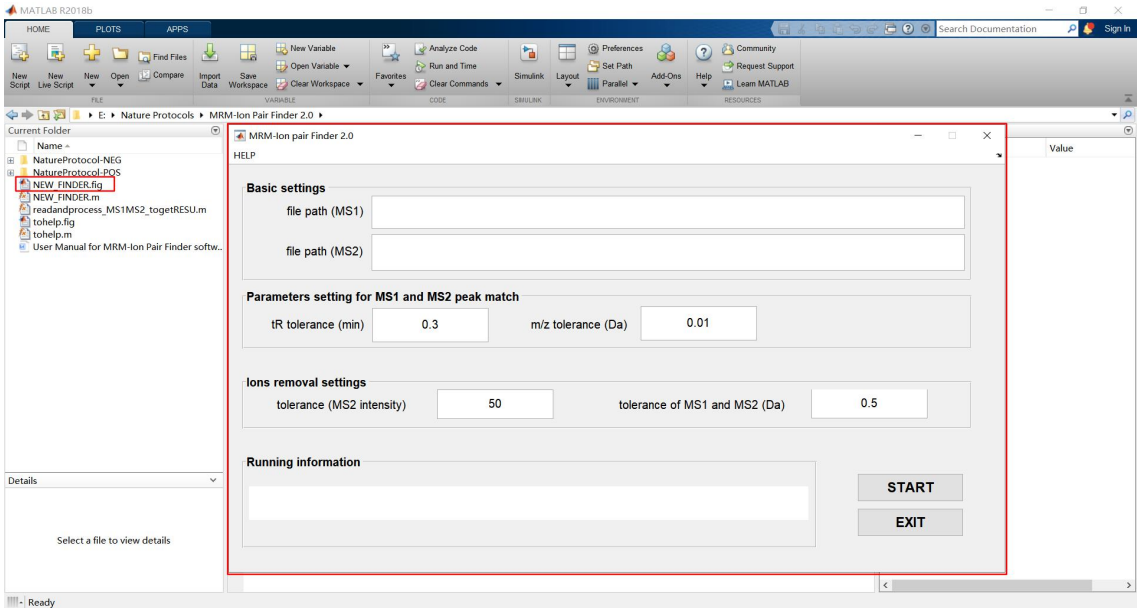

Figure 3 | User interface of MRM-Ion Pair Finder v2.0

6 According to the specific experimental situation, set the software parameters, click "START" to start running the program. The functions of each parameter are shown in **Table 1**.

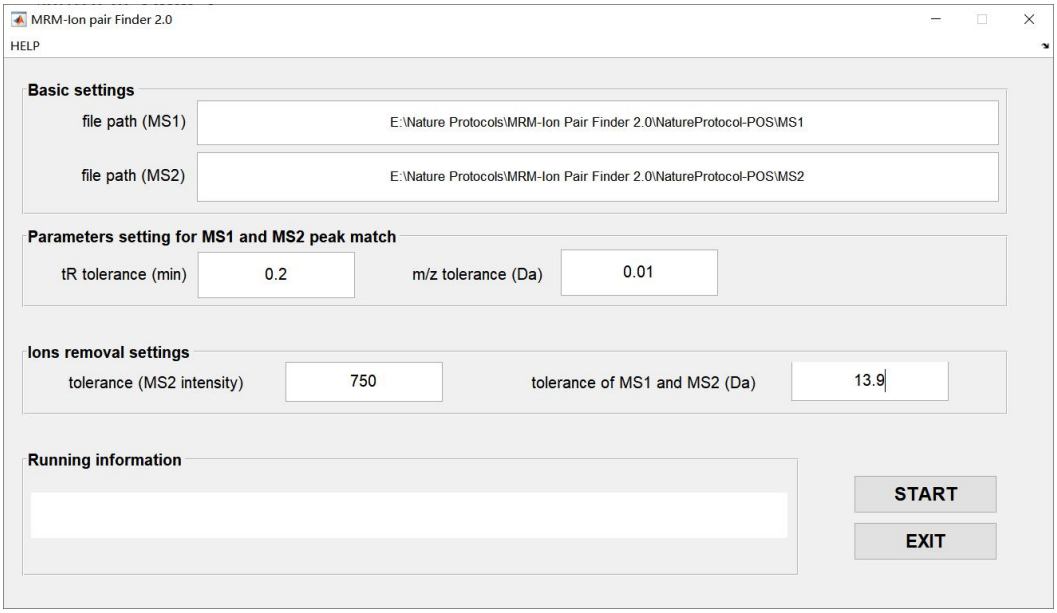

Figure 4 | Parameter setting.

7 The result (csv file) is automatically saved under the subfolder named with the software run-end time.

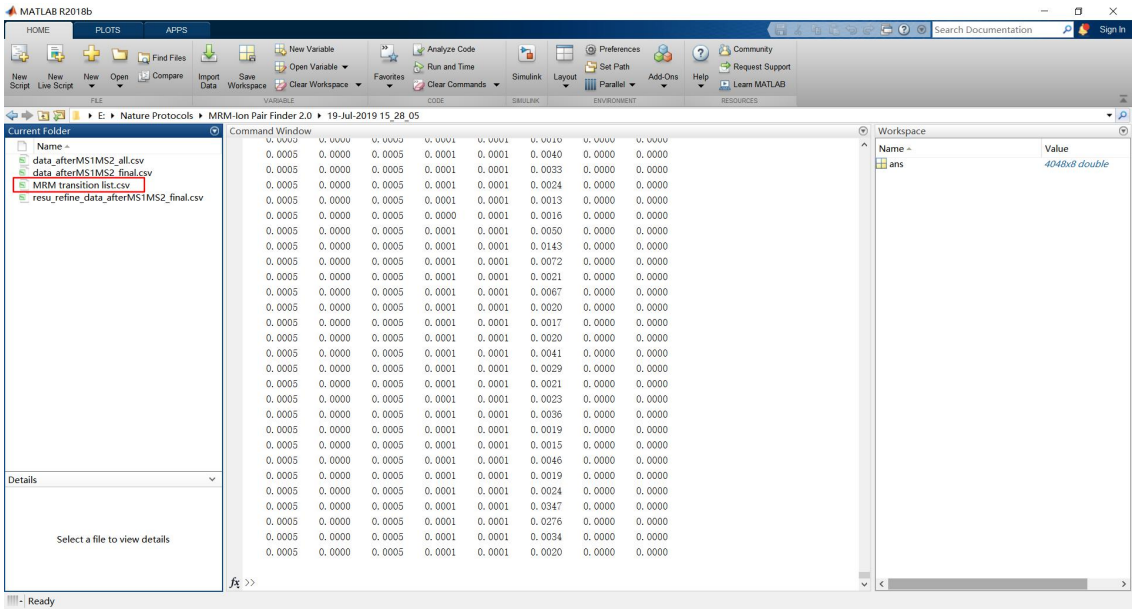

Figure 5 | Result of MRM-Ion Pair Finder v2.0

Table 1. MRM-Ion Pair Founder parameter meaning

Parameters	Meaning
file path (MS1)	MS1 and MS2 file path. Notice：MS1 file format must be same as **Figure 6**, MS2 file must name as CE voltage such as 15V.mgf, 30V.mgf and 45V.mgf. The two folders are separate, and there should be no other files under the folders.
file path (MS2)	
tR tolerance (min)	tr tolerance between MS1 and MS2 files
m/z tolerance (Da)	m/z tolerance between MS1 and MS2 files
tolerance (MS2 intensity)	If the response of product ion is smaller than threshold, it will be deleted.
tolerance (@MS1~MS2)	If the m/z difference between precursor and produce ion is smaller than tolerance (MS1~MS2), it will be deleted.

	A	B	C	D	E
1	mz	rt	SPC.15V.1	SPC.30V.1	SPC.45V.1
2	54.00938845	867.868	297220.096	262190.9618	290320.048
3	54.00912905	1757.91	625295.7788	57753.50294	508164.8606
4	54.00921987	54.927	235518.085	324180.104	301519.82
5	54.01119574	523.842	475525.3446	445851.4657	181282.3557
6	54.00919802	789.403	129355.0893	117460.656	99407.418
7	54.01136902	1304.41	129159.045	138729.1211	157347.0019
8	54.00933384	970.782	194203.1457	229080.5087	347223.7371
9	54.01844932	433.3335	54042.9906	14920.67167	102687.789
10	54.01759992	522.368	84113.67375	328847.1766	115624.6623
11	54.01697932	1754.56	170126.009	279204.228	127874.7947
12	54.01641829	55.7585	110503.624	111037.9344	99098.39394
13	55.0567792	60.757	28752.68	25478.66413	12446.74844
14	55.93630528	1750.68	31140.26222	42821.482	18110.71833
15	56.0523362	60.757	10795.76394	18967.00378	13244.78276
16	58.0679415	46.5375	20838.0095	12620.58833	16900.39632
17	144.102	48.117	38967.66568	31806.88792	85035.70508
18	61.04293185	48.957	37976.34894	32376.92981	9016.108889
19	158.0235658	524.119	506317.2438	667980.866	803071.7549
20	68.01188815	50.411	43350.42776	46757.788	45180.02439
21	68.01296893	1756.18	88262.62316	196798.1131	121413.1139
22	68.01177128	855.528	36361.88345	46640.264	63416.93967
23	68.01537289	210.391	53380.03132	14512.732	25000.23589
24	68.01540104	1771.88	148018.255	52551.85034	41346.363
25	69.07150265	68.896	13862.86433	17983.69462	20762.275
26	70.06726828	48.117	28371.84375	17861.49933	61813.95692
27	71.93055848	1753.54	44392.98429	30811.89	57766.65146
28	72.08374194	60.11	10626.13255	39162.84276	55824.4763

Figure 6 | MS1 format for MRM-Ion Pair Finder v2.0. The first column must be m/z and the second column must be retention time. Intensity of each samples should be start from third column.