

## Quick Start Guide for BAFinder 2.0

### To start

For Windows64 system:

Open folder “BAFinder\_windows64”, and double click “BAFinder\_win64.exe”.

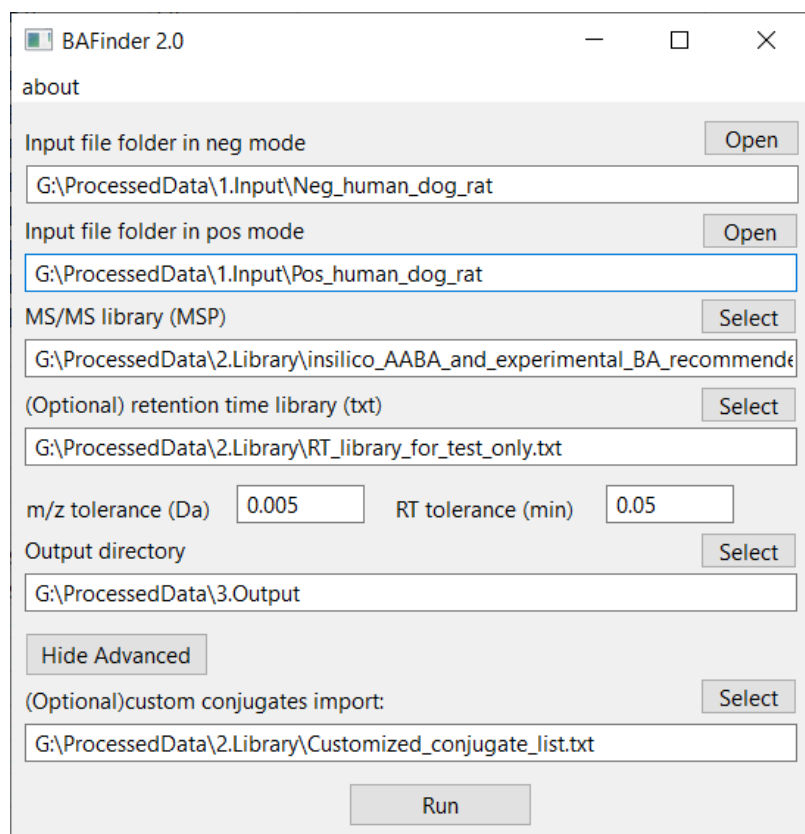
For macOS system:

Open folder “BAFinder\_macosx”, in the sub-directory “Contents/MacOS”, find BAFinder\_macosx and double click it.

### Work with test data

Select the test data folder and library files, enter the parameters as shown in the screenshot below and click on the “Run” button. A processing bar will show up. The output should be same as the files in “3. Output \Result\_for\_test\_data” folder. It takes about 30 min on a PC with Intel(R) Xeon(R) W-2235 CPU and 64 GB RAM.

Open the “Final\_summary.csv” file to check the bile acids annotated. After filtering the annotation (“-BA” to get amino acid conjugated bile acids), confidence level (remove blank) and sample numbers (detected in at least 2 samples in one species), the result should be same as the Table S3 in the manuscript.



BAFinder 2.0

about

Input file folder in neg mode

G:\ProcessedData\1.Input\Neg\_human\_dog\_rat

Input file folder in pos mode

G:\ProcessedData\1.Input\Pos\_human\_dog\_rat

MS/MS library (MSP)

G:\ProcessedData\2.Library\insilico\_AABA\_and\_experimental\_BA\_recommende

(Optional) retention time library (txt)

G:\ProcessedData\2.Library\RT\_library\_for\_test\_only.txt

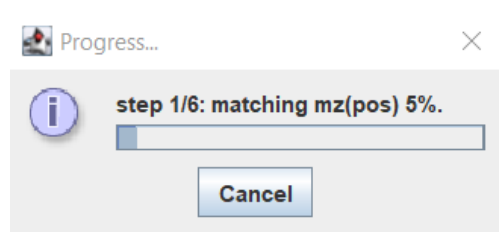
m/z tolerance (Da)  RT tolerance (min)

Output directory

G:\ProcessedData\3.Output

(Optional) custom conjugates import:

G:\ProcessedData\2.Library\Customized\_conjugate\_list.txt



Progress...

step 1/6: matching mz(pos) 5%.

### Work with your own data

Most operations of BAFinder 2.0 are consistent with the previous version. To prepare your input data files, please refer to the User Manual of BAFinder at <https://bafinder.github.io/>.