

# SmallP Manual

Version 2024.12

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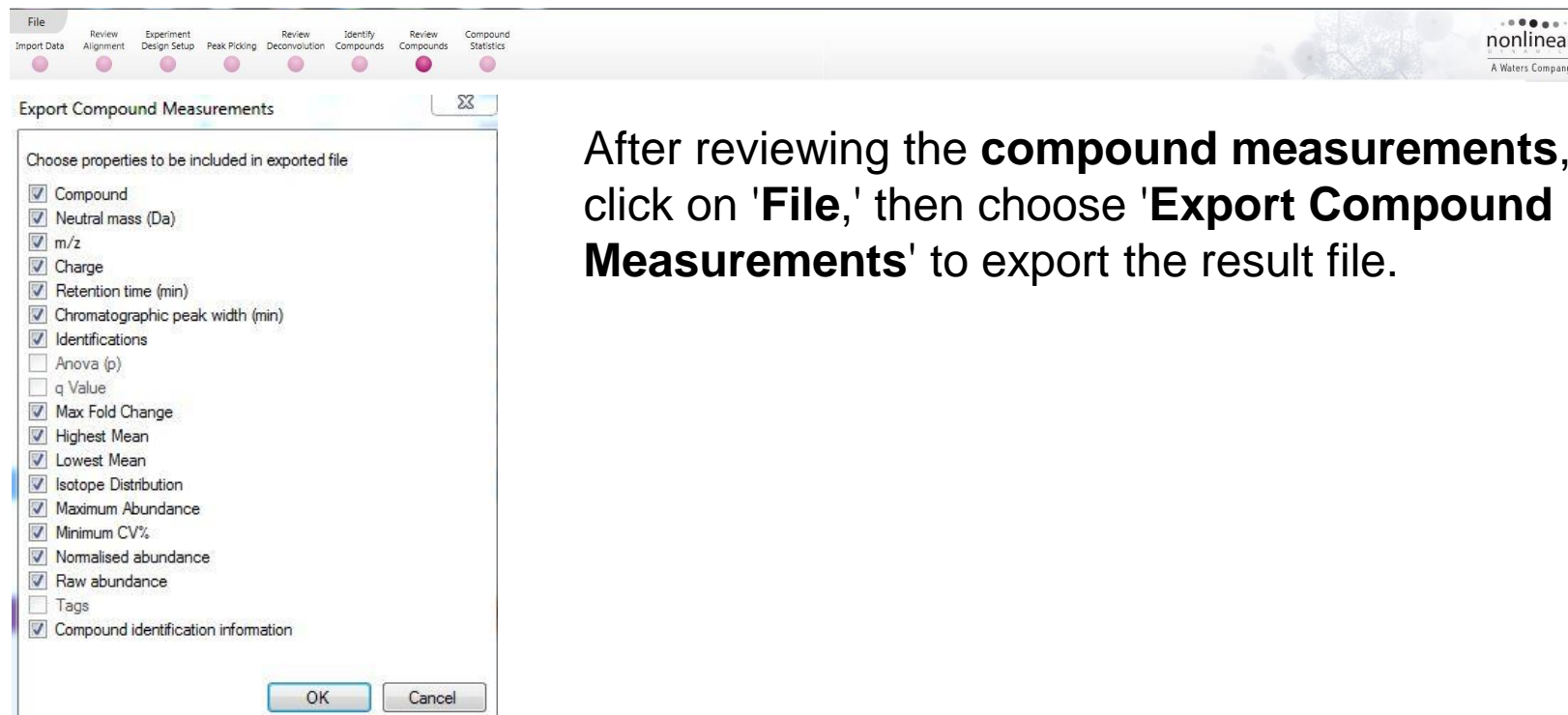
5. Run

# 1. Prepare files: Progenesis QI

Progenesis QI can deal with massive datasets from multiple instruments for spectral deconvolution in both GC/MS and data-independent MS/MS.

Progenesis QI tutorial:

[https://www.waters.com/webassets/cms/promotion/media/designed\\_page/local\\_china\\_common/progenesis\\_qi/#Progenesis](https://www.waters.com/webassets/cms/promotion/media/designed_page/local_china_common/progenesis_qi/#Progenesis)



# 1. Prepare files: MS-DIAL



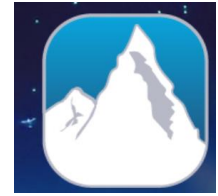
MS-DIAL

MS-DIAL is a **free** application that supports multiple instruments for spectral deconvolution in both GC/MS and data-independent MS/MS. MS-DIAL5 tutorial: <https://systemsomicslab.github.io/msdial5tutorial/>

The screenshot displays the MS-DIAL software interface. The top menu bar includes Processing, Data visualization, Search, View, Option, Export, Help, and Filter. Below the menu is a toolbar with icons for Peak list result, Alignment result, Export normalization result, Molecular spectrum networking export, and Parameter export (Tab-delimited text). A red box highlights the 'Peak list result' icon in the toolbar. The main window is divided into several panels: 'File navigator' on the left, 'Peak spot navigator' with a 'Label' dropdown and 'Peak spots: 100% Num. 14029', 'Eic of focused spot' showing a chromatogram, 'Structure' and 'Candidates' tables, 'Survey scan (MS1) spectrum', 'Peak spot viewer', 'Deconvolution vs. Reference' plot, and 'Measurement' plot. A red arrow points from the 'Peak list result' icon to the 'Peak list export' dialog box. The dialog box has a 'Directory' field with a 'Browse' button. Below are two empty boxes for 'Available files to export' and 'File to be exported', with buttons 'Add all >>', 'Add ->', '<- Remove', and '<< Remove all'. At the bottom, there is a section for 'Peak table' export with a checked checkbox, 'Export format: txt', 'Spectra type: deconvoluted', 'Isotope region: 2', and options for 'Nist format (\*.msp) deconvoluted', 'MASCOT format (\*.mgf) deconvoluted', and 'MassBank record' with a 'ContributorID' field. 'Export' and 'Cancel' buttons are at the bottom right.

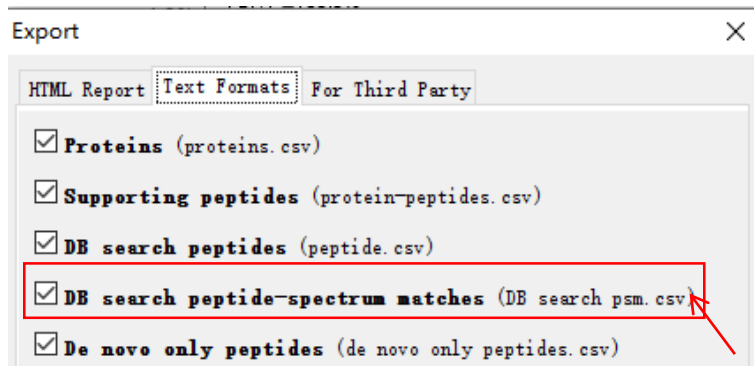
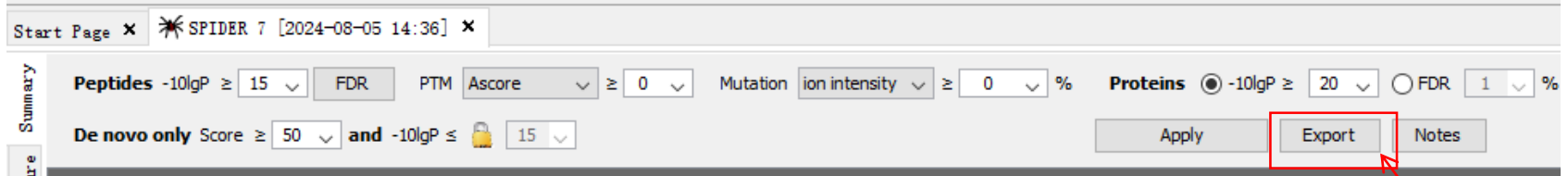
After completing the data import, click on '**Peak List Result.**' Then, export the result as a .txt file.

# 1. Prepare files: Peaks studio



PEAKS Studio is a software platform with complete solutions for discovery and targeted proteomics.

PEAKS Studio tutorial: <https://www.bioinfor.com/peaks-studio/>



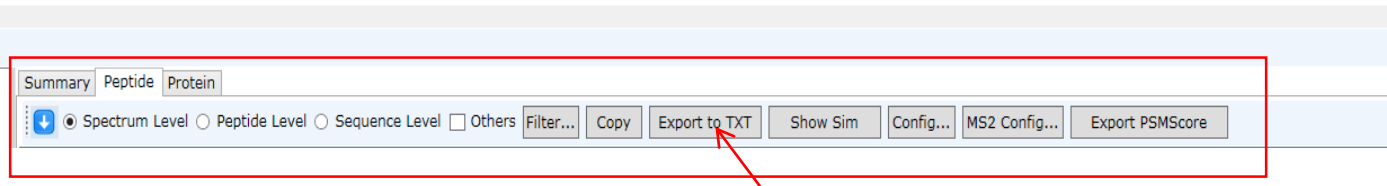
After completing the data processing, click on '**Export**'. Then, Click the **DB search peptide-spectrum matches**.

# 1. Prepare files: pFind



pFind Studio is a **free** software platform with complete solutions for discovery and targeted proteomics.

pFind Studio tutorial: <http://pfind.org/software/pFind/index.html>

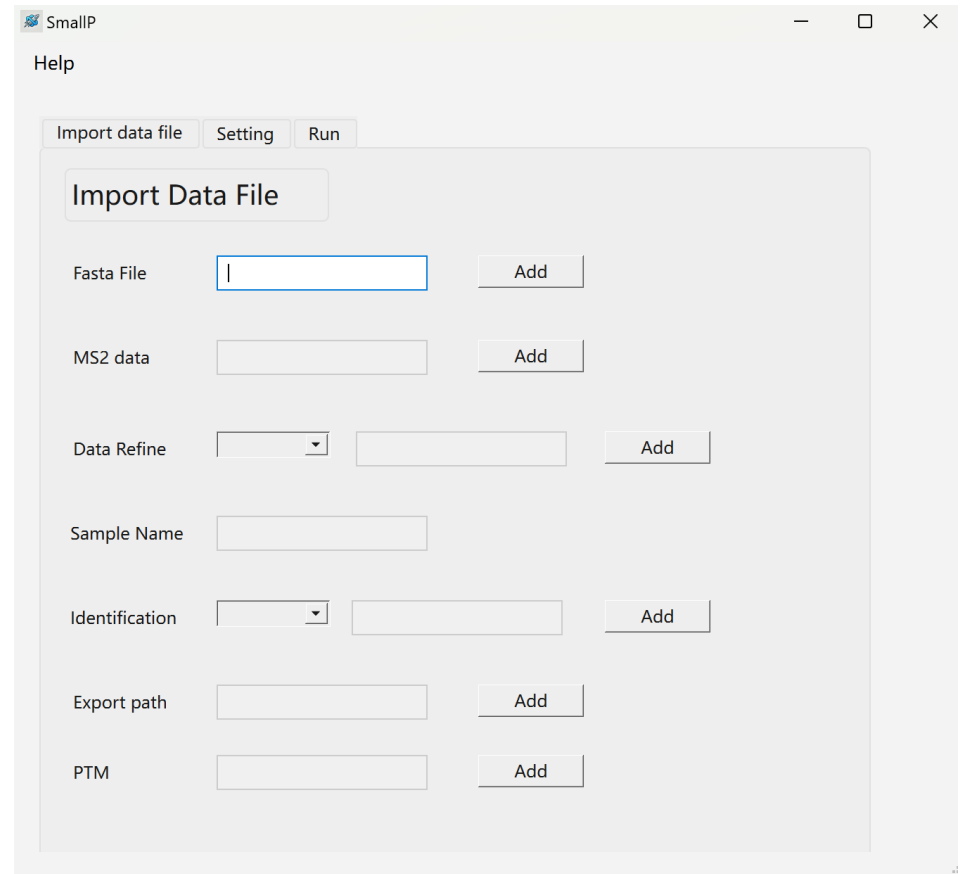


After completing the data processing, it will turn to pbuild program. Click on '**peptide**'. Then, Click the **Export to TXT**.

# 2. Start up

Small P is a free application intended solely for academic use. The program is easy to use for identifying disulfide bonds in peptides. By running Smallp.exe, you can import data, set parameters, and view the results.

For more detailed instructions:



# 3.Upload datafiles: Database

SmallP

Help

Import data file Setting Run

Import Data File

- 1 Fasta File  Add
- 2 MS2 data  Add
- 3 Data Refine  Add
- 4 Sample Name
- 5 Identification  Add
- 6 Export path  Add
- 7 PTM  Add

The software requires some profiles to function properly.

**Fasta File:** Click the “Add” button to select **a fasta file (.fasta)** as the input file. The fasta file should correspond to the search files you will upload subsequently.



# 3.Upload datafiles: mgf documents

SmallP


Help


Import data file Setting Run

Import Data File

- 1 Fasta File  Add
- 2 MS2 data  Add
- 3 Data Refine  Add
- 4 Sample Name
- 5 Identification  Add
- 6 Export path  Add
- 7 PTM  Add

**MS2 Data:** Click the “Add” button to select the folder path containing **the input file (.mgf)**. The file name should be formatted as **xxx-Nat-num**, **xxx-RA-num**, or **xxx-RAE-num**.

 TQY-Nat-10.mgf

 TQY-RA-10.mgf

File naming example

# 3.Upload datafile: Peak detection

SmallP

Help

Import data file Setting Run

Import Data File

1 Fasta File  Add

2 MS2 data  Add

3 Data Refine  Add

4 Sample Name

5 Identification  Add


6 Export path  Add


7 PTM  Add

MSDIAL  
QI

**Data Refine:** Click the “Add” button to select the folder path containing the results files from **MSDIAL** or **QI**. The drop-down box allows you to select software. These files could provide the MS information.

**See the next section for detailed file export guidelines.** The file name should be formatted as **xxx-Nat-num**, **xxx-RA-num**, or **xxx-RAE-num**.

 TQY-Nat-10

 TQY-RA-10

File naming example

[MSDIAL file export](#)


[QI file export](#)


# 3.Upload datafile: Sequence identification

The screenshot shows the 'SmallP' application window with the 'Import Data File' dialog open. The dialog has tabs for 'Import data file', 'Setting', and 'Run'. The 'Import Data File' tab is active, showing a list of fields for data import:

- 1 Fasta File: Text input field with an 'Add' button.
- 2 MS2 data: Text input field with an 'Add' button.
- 3 Data Refine: A dropdown menu and a text input field, with an 'Ac' button.
- 4 Sample Name: Text input field.
- 5 Identification: A dropdown menu with a red box around it and a red arrow pointing to the 'peaks' and 'pfind' options in the dropdown list. There is also an 'Ac' button next to it.
- 6 Export path: Text input field with an 'Add' button.
- 7 PTM: Text input field with an 'Add' button.

**Identification:** Click the “Add” button to select the folder path containing the results files from **Peaks or pFind**. The drop-down box allows you to select software. These files could provide the peptide identification information. **See the next section for detailed file export guidelines.** The file name should be formatted as **xxx-Nat-num, xxx-RA-num, or xxx-RAE-num**.

 TQY-Nat-10

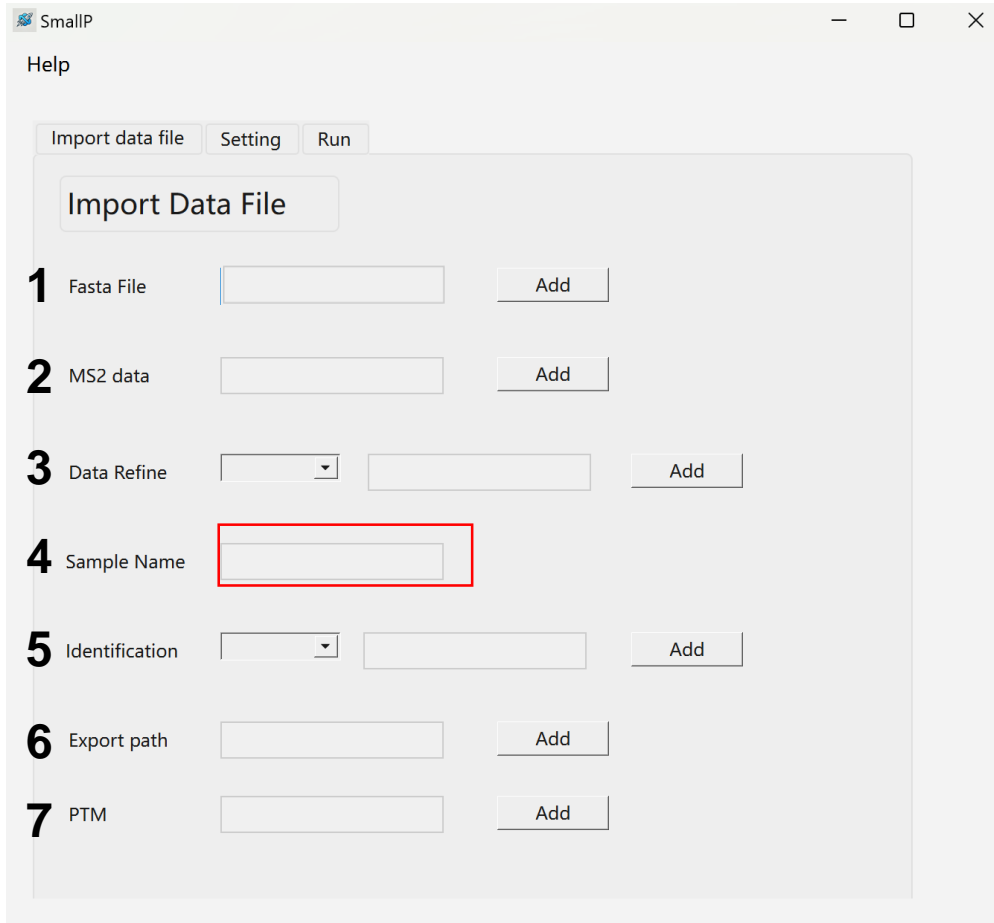
 TQY-RA-10

File naming example

[peaks file export](#)

[pfind file export](#)

# 3.Upload datafile: Peak detection



SmallP


Help


Import data file Setting Run

Import Data File

- 1 Fasta File  Add
- 2 MS2 data  Add
- 3 Data Refine  Add
- 4 Sample Name
- 5 Identification  Add
- 6 Export path  Add
- 7 PTM  Add

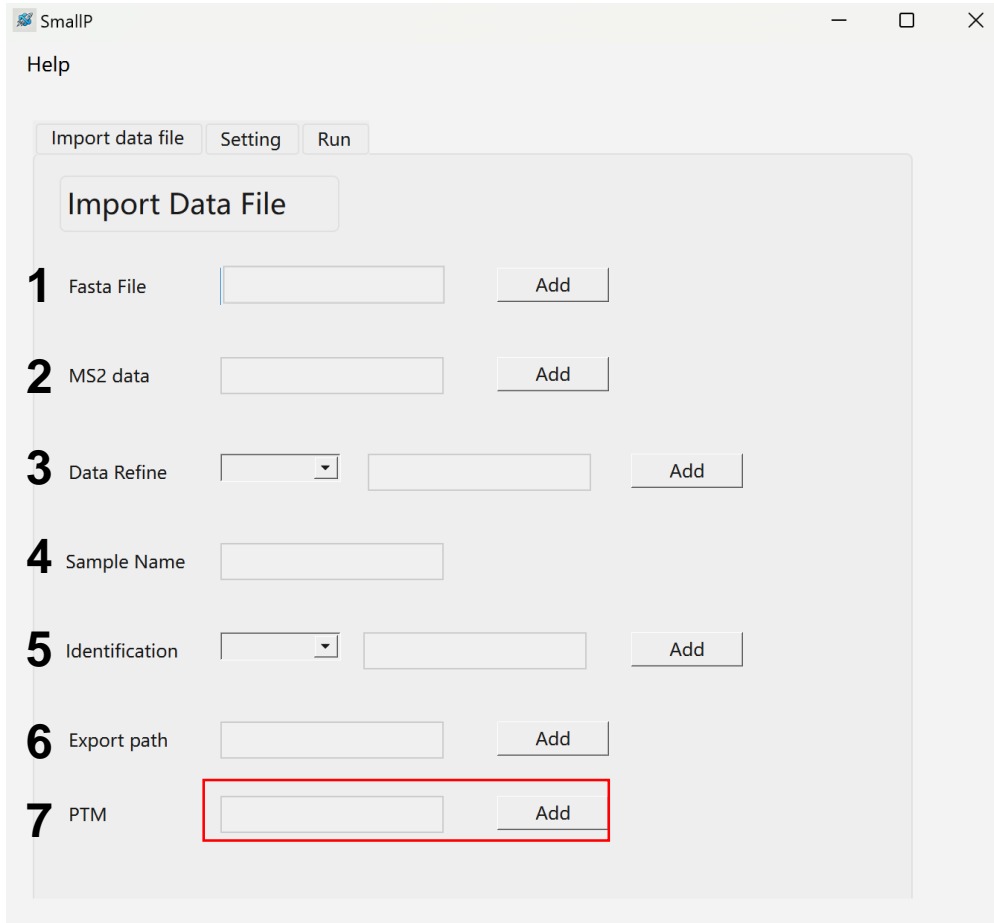
**Sample Name:** The file name should be formatted as **xxx-Nat-num, xxx-RA-num, or xxx-RAE-num**. The sample name should be in the **format xxx**.

 TQY-Nat-10

 TQY-RA-10

If the files are formatted as shown in the figure, the sample name should be “TQY”.

# 3.Upload datafile: PTM file



SmallP

Help

Import data file Setting Run

Import Data File

- 1 Fasta File  Add
- 2 MS2 data  Add
- 3 Data Refine  Add
- 4 Sample Name
- 5 Identification  Add
- 6 Export path  Add
- 7 PTM  Add

**PTM:** Click the “Add” button to select the file that contains the post-translational modification information (.xlsx).

# 4. Setting

SmallP

Help

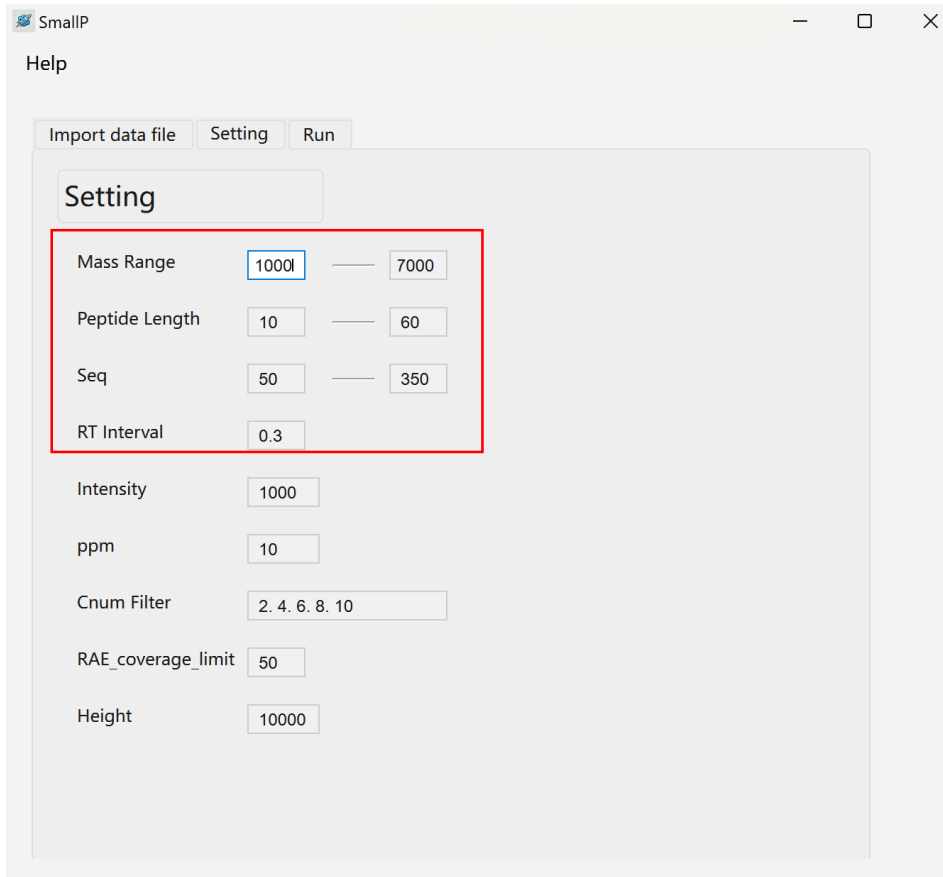
Import data file Setting Run

Import Data File

- 1 Fasta File  Add
- 2 MS2 data  Add
- 3 Data Refine  Add
- 4 Sample Name
- 5 Identification  Add
- 6 Export path  Add
- 7 PTM  Add

**Export path:** Click the “Add” button to select the folder path where the final result files from the Smallp software will be saved.

# 4. Setting



**Setting:** There are some default parameters you can change depending on your experiment design.

- **Mass range:** The range of mass to be analyzed.
- **Peptide length:** The range of peptide length to be analyzed in the database.
- **Seq:** The range of peptide length to be analyzed in the FASTA file.
- **RT:** The maximum retention time

# 4. Setting

SmallIP

Help

Import data file Setting Run

Setting

Mass Range 1000 7000

Peptide Length 10 60

Seq 50 350

RT Interval 0.3

Intensity 1000

ppm 10

Cnum Filter 2. 4. 6. 8. 10

RAE\_coverage\_limit 50

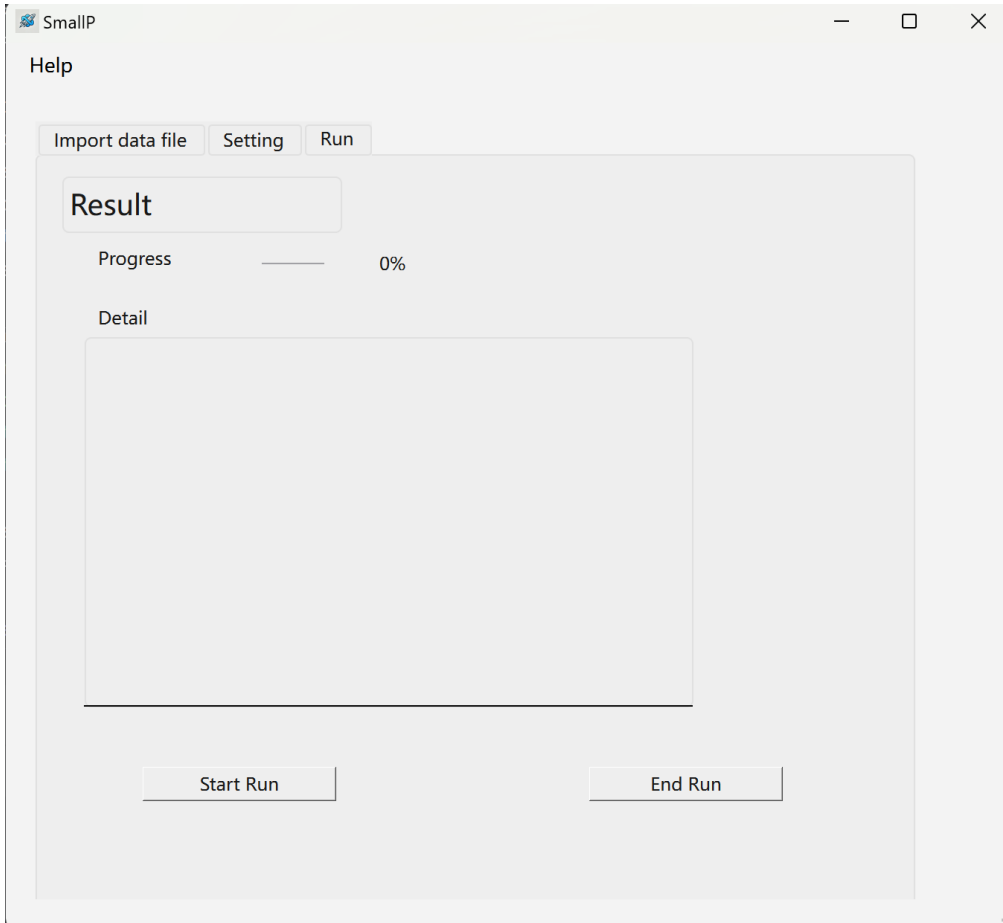
Height 10000

**Setting:** There are some default parameters you can change depending on your experiment design.

- **Intensity limit:** The minimum mass intensity in the data refine files.
- **Cnum filter:** The number of disulfide bonds you would like to search for. The number should be divided with",".
- **RAE coverage limit:** The minimum coverage of RAE.
- **Height:** The minimum ion intensity in the peptide identification files.



# 5.Run



Click the “**Start Run**” button to initiate the program. The progress bar will indicate the progress rate from 0 to 100. The results will be displayed in the detail window. If an error occurs, you can click the “**End Run**” button to stop the program and rerun it. There are totally 7 steps during this programs.