OpenSpec Manual

Version.2025

Demo Data

The data was downloaded from previous publications[1]. To facilitate the use and testing of the tool, the relevant files have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX[2, 3] partner repository with the dataset identifier **PXD063366**. The link to the dataset in iProx: https://www.iprox.cn/page/project.html?id=IPX0011827000

[1] Guzman UH, et al. (2024) Ultra-fast label-free quantification and comprehensive proteome coverage with narrow-window data-independent acquisition. Nat Biotechnol. 2024;42(12):1855-66.
[2] Ma J, et al. (2019) iProX: an integrated proteome resource. Nucleic Acids Res. 47(D1), D1211-D1217.
[3] Chen T, et al. (2021) iProX in 2021: connecting proteomics data sharing with big data. Nucleic Acids Res. 50(D1): D1522-D1527.

CONTENTS

Software Download

1 pFind Download

Downloads

pFind 3.1.6 for windows (64bit)

- released on Jan. 02, 2020 -

Please click here to download the user manual

For license application, please visit i.pfind.org.

For technical support, please visit forum.pfind.org.

For other issues, please contact support@pfind.org.

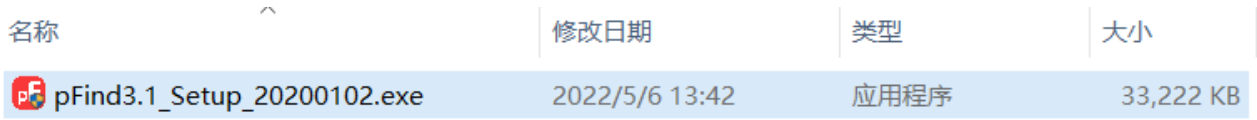
Notice:

* The expiration date is set on **Jan. 10, 2023**.

** **尊敬的用户您好，pFind 3.1.6此次更新并不影响此前的license使用。您在下载安装之后，可以直接导入此前申请的license文件，而不用再次申请。谢谢！**

** **License since version 3.1.3 is still valid for this version. After downloading and installing this version, you can just import the license applied previously instead of applying a new license. Thank you.**

* .NET framework 4.5 or a higher version is required.

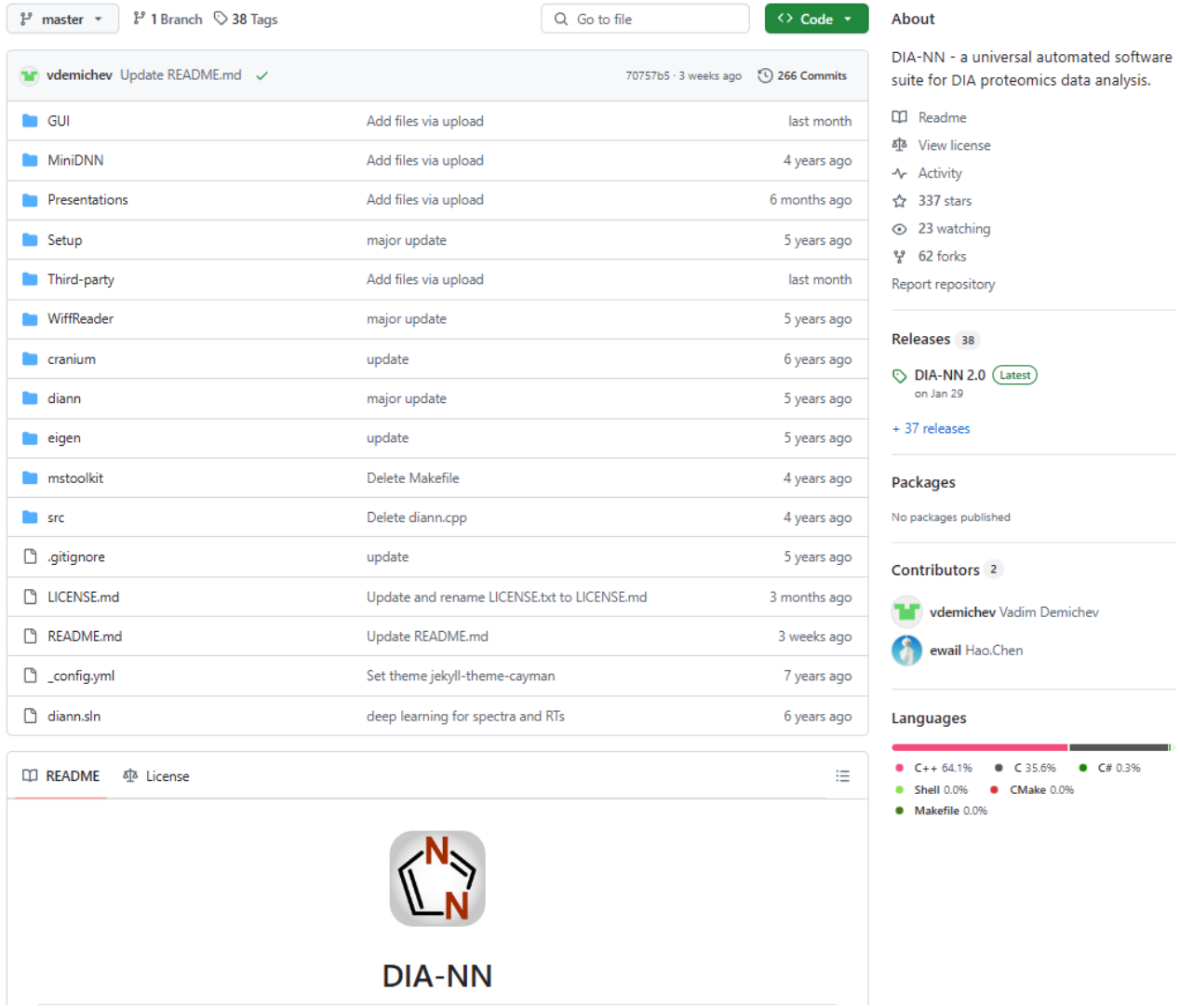


Login

http://pfind.net/software/pFind/index.html#Downloads

and download pFind3 at the bottom of the page.

2 DIA-NN Download



Login https://github.com/vdemichev/DiaNN and download **the latest version** at the top of the page.

3 PepPre Download

Release

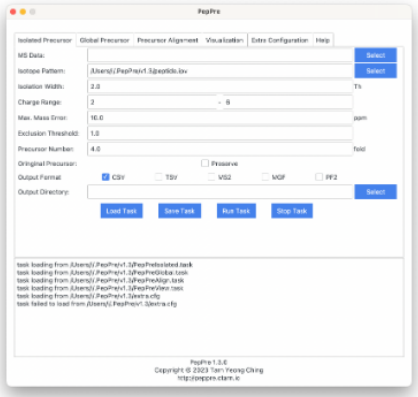
PepPre 1.3.0 2023-12-29

Linux | macOS (pkg) | macOS (zip) | Windows

🎉 Our paper "PepPre: Promote Peptide Identification Using Accurate and Comprehensive Precursors" has been accepted by *Journal of Proteome Research*!

Note:

1. add experimental features for global peptide precursor analysis.
2. support .mes input, which is a binary format and is much faster than text-based formats.
3. able to split output into batches to support large data from instrument such as Astral.
4. support .pf2 output, which is commonly used by software from pFind Team.
5. improved UI.



PepPre 1.2.0 2023-5-7

Linux | macOS (pkg) | macOS (zip) | Windows

Note:

1. improved UI.

PepPre 1.1.1 2023-4-20

Linux | macOS (pkg) | macOS (zip) | Windows

Note:

1. change path for saving configuration.

PepPre 1.1.0 2023-4-19

Linux | macOS | Windows

Note:

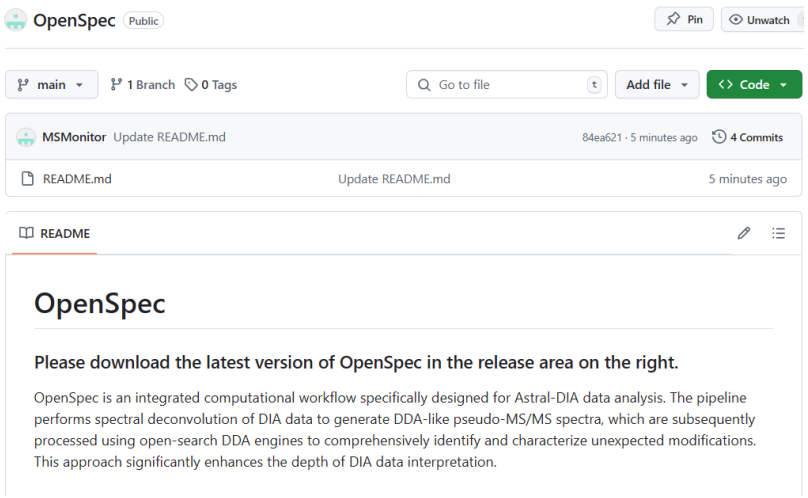
1. embed `PepPepView` which can display deisotoped precursor ions and corresponding identification.
2. adjust arguments of CLI interface.

Login https://peppre.ctarn.io/

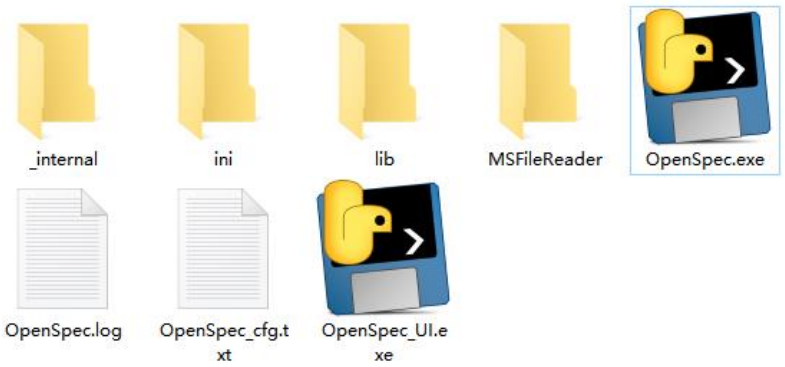
and download **PepPre** at the top of the page.

3 OpenSpec Download

①



②



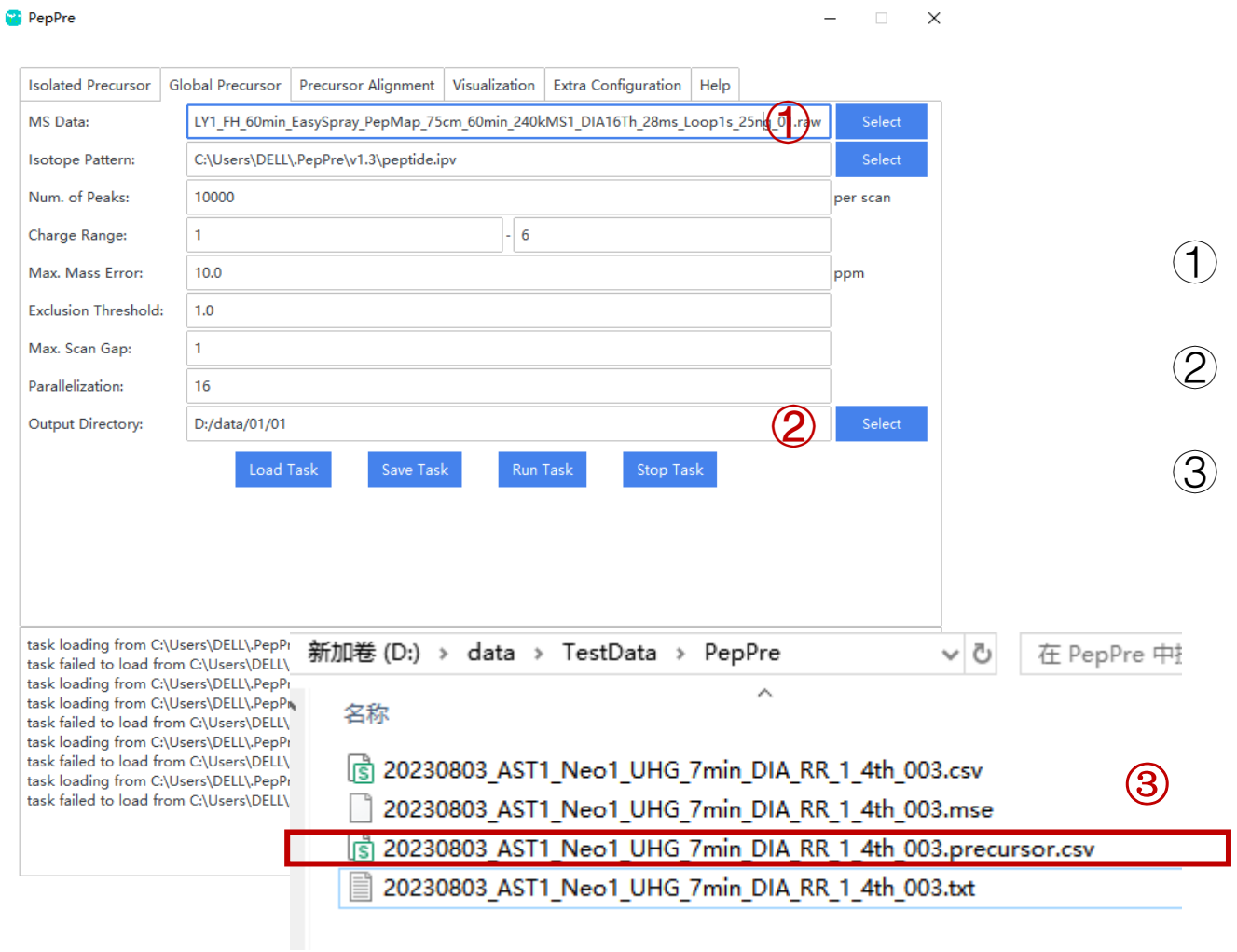
① Login https://github.com/BUAA-LiuLab/OpenSpec.git and download **the latest version** at the top of the page.

② Double click **OpenSpec_UI.exe to analyze.**

OpenSpec Manual for

Pseudo-MS/MS Spectra Generation

1 Extract MS Features with PepPre

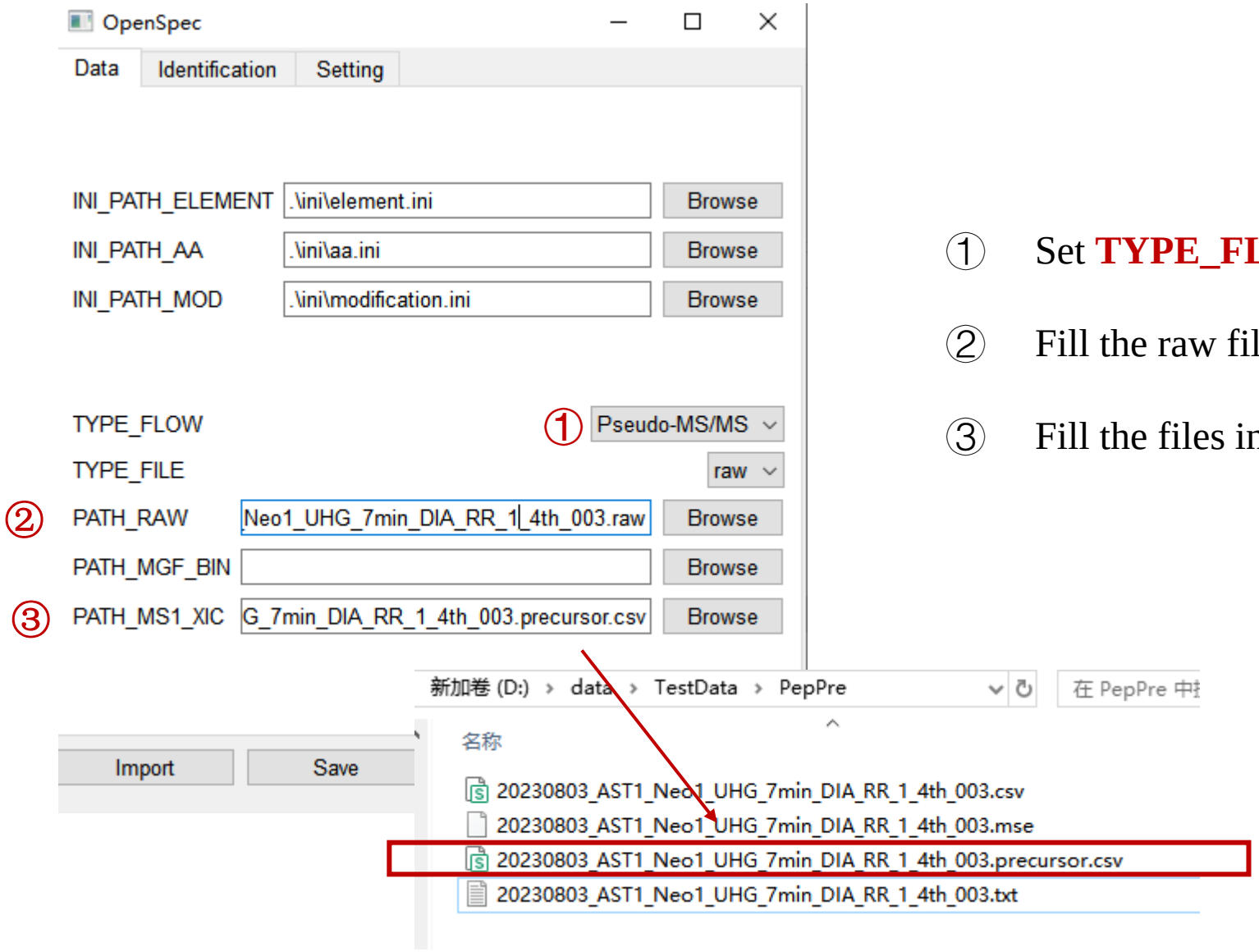


① Load raw file.

② Select output path.

③ The result file for subsequent analysis.

2 Pseudo-MS/MS Spectra Generation

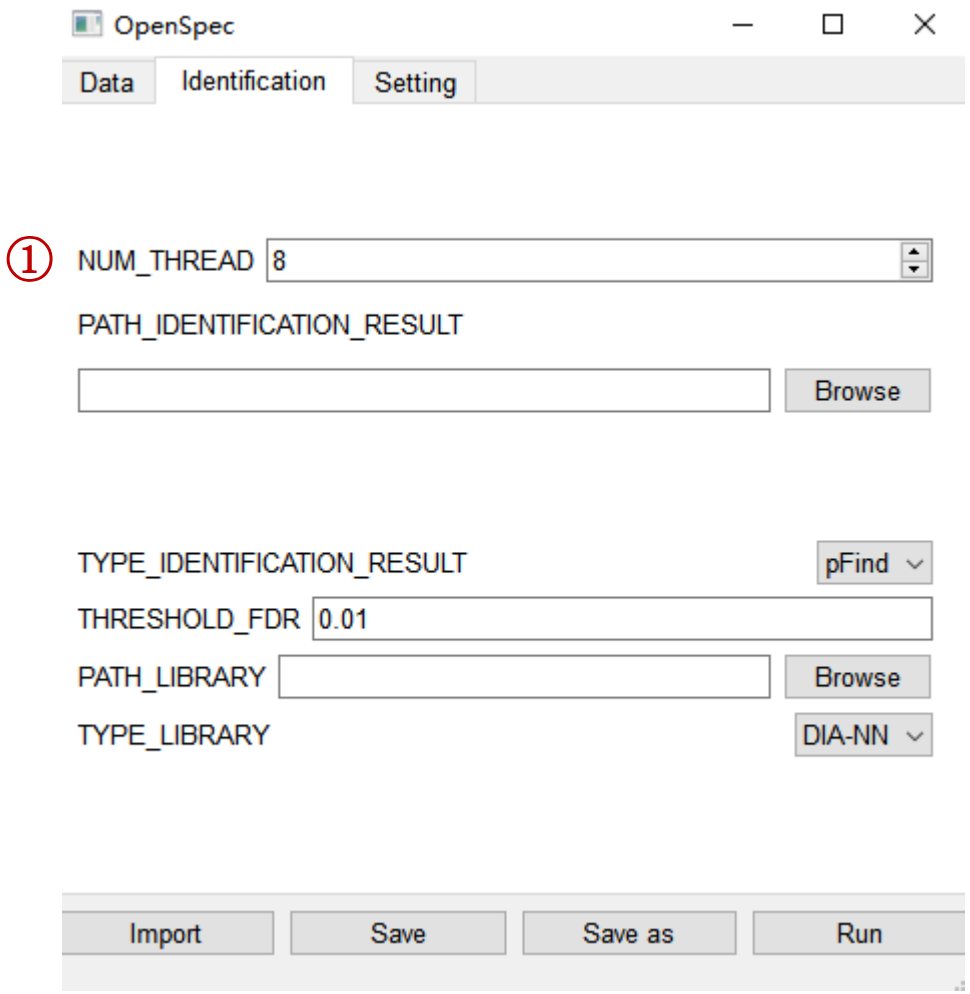


① Set **TYPE_FLOW** as **Pseudo-MS/MS**.

② Fill the raw files into the **PATH_RAW**.

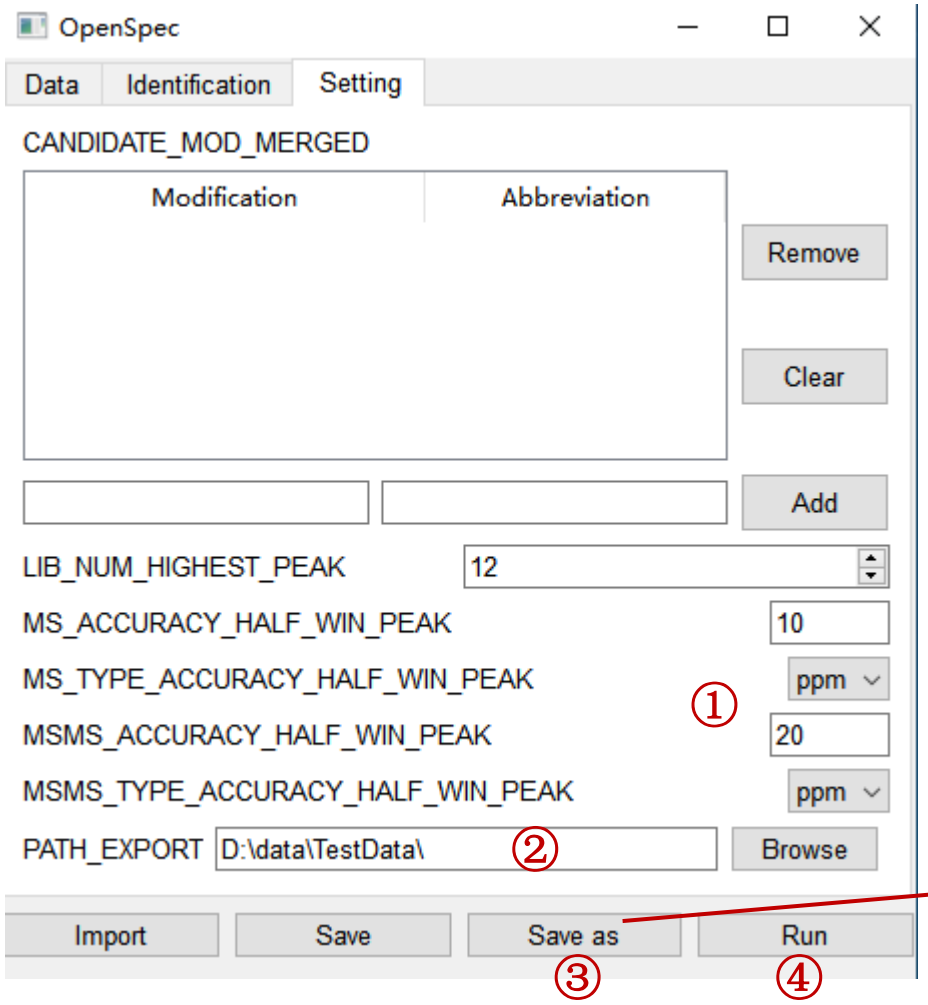
③ Fill the files into the **PATH_MS1_XIC**.

2 Pseudo-MS/MS Spectra Generation



① Adjust **NUM_THREAD** according to your computer's performance; the default is 8.

2 Pseudo-MS/MS Spectra Generation

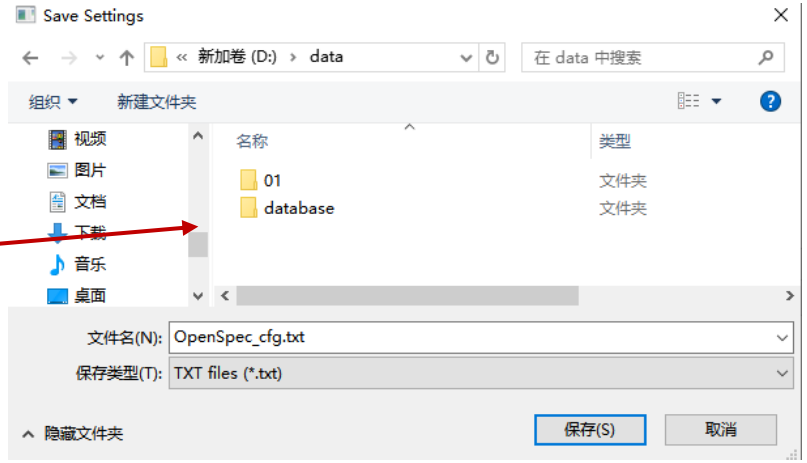


① Adjust the MS/MSMS accuracy based on your needs.

② Set the **PATH_EXPORT** for saving the results.

③ Click **Save as** button to save the config file.

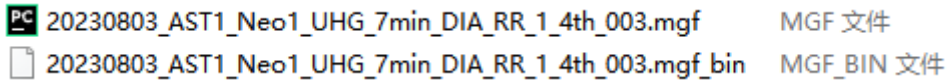
④ Click **Run** button to start.



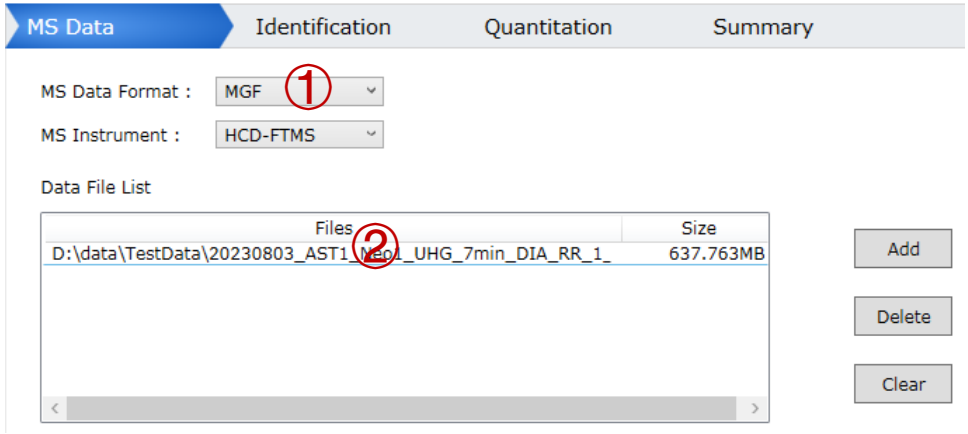
2 Pseudo-MS/MS Spectra Generation

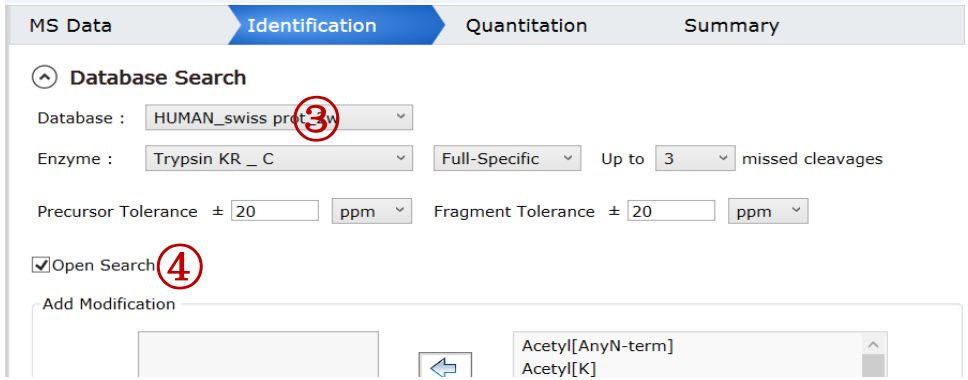
```
[OpenSpec]      Checking the environment...

[OpenSpec]      Reading ini files...

[OpenSpec]      Extracting raw files...

[OpenSpec]      Extract RAW File: D:\data\TestData\20230803_AST1_Neo1_UHG_7min_DIA_RR_1_4th_003.raw
2025/4/27 20:37:07
[INFO] Checking File D:\data\TestData\20230803_AST1_Neo1_UHG_7min_DIA_RR_1_4th_003.raw

2025/4/27 20:37:07
[INFO] Reading scan titles from D:\data\TestData\20230803_AST1_Neo1_UHG_7min_DIA_RR_1_4th_003.raw

2025/4/27 20:37:13
[INFO] Writing all spectrum to D:\data\TestData\20230803_AST1_Neo1_UHG_7min_DIA_RR_1_4th_003.bin

2025/4/27 20:37:20
[INFO] Finish parsing file D:\data\TestData\20230803_AST1_Neo1_UHG_7min_DIA_RR_1_4th_003.raw


[OpenSpec]      Preprocessing...
0 / 660
0 / 81913
10000 / 81913
20000 / 81913
30000 / 81913
40000 / 81913
50000 / 81913
60000 / 81913
```

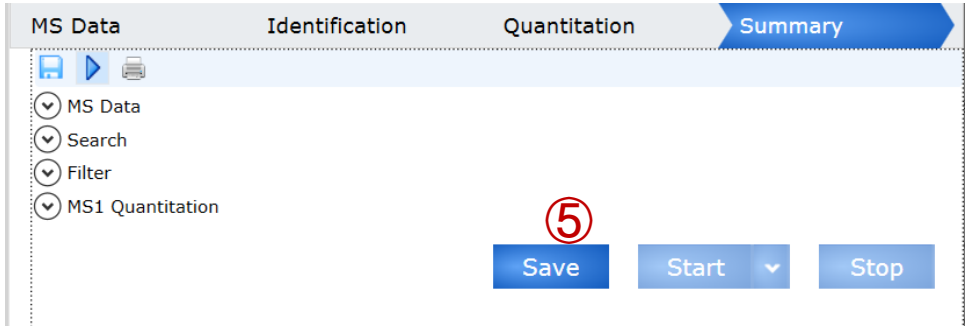
① The result files.

① 

3 Open Search with pFind3







① Set **MS Data Format** to **MGF**.

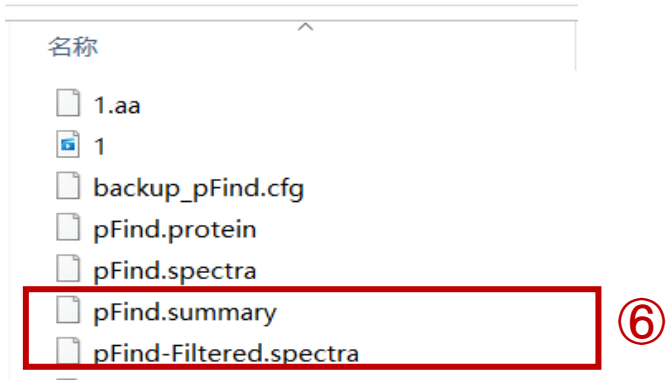
② Add **mgf file**.

③ Set the fasta database.

④ Set to **Open Search** is better.

⑤ Click **Save** and **Start** buttons, the progress information will be shown in Output interface.

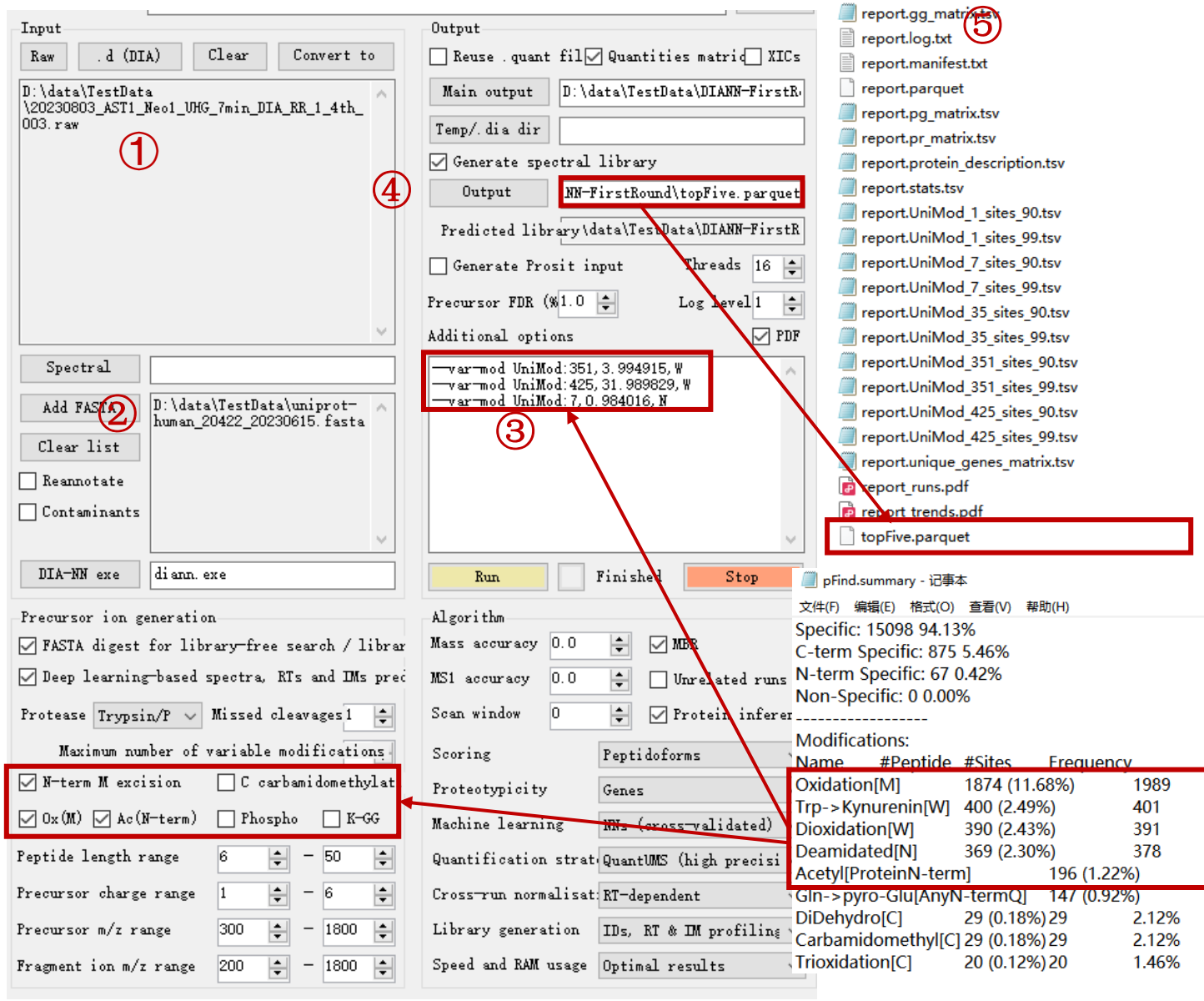
⑥ The identification result files are used for hybrid spectral library construction.



OpenSpec Manual for

Hybrid Spectral Library Construction

1 First-Round Search with DIA-NN.



① Load raw file.

② Set the fasta database.

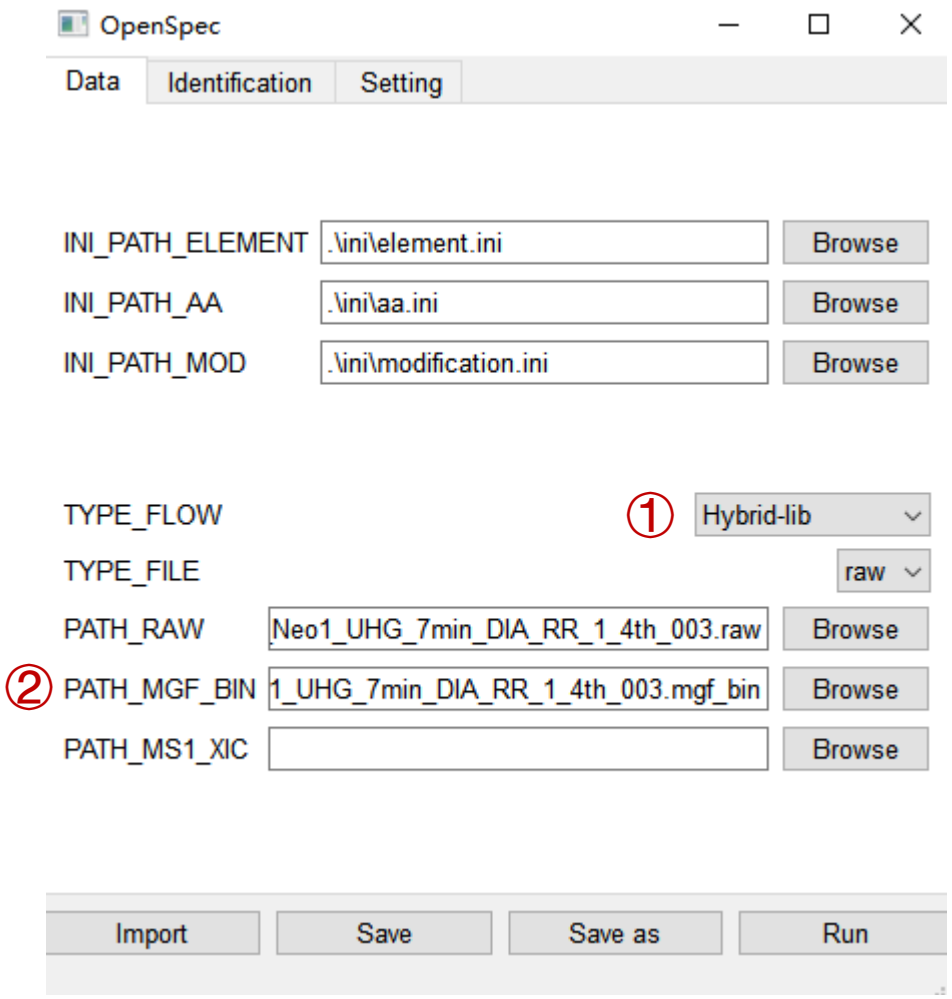
③ Set the **high-abundance modifications** according to open search results.

④ Select output path.

⑤ The identification result files are used for hybrid spectral library construction.

Note: since DIA-NN does not support certain symbols (e.g., ->), accession of UniMod are used uniformly instead.

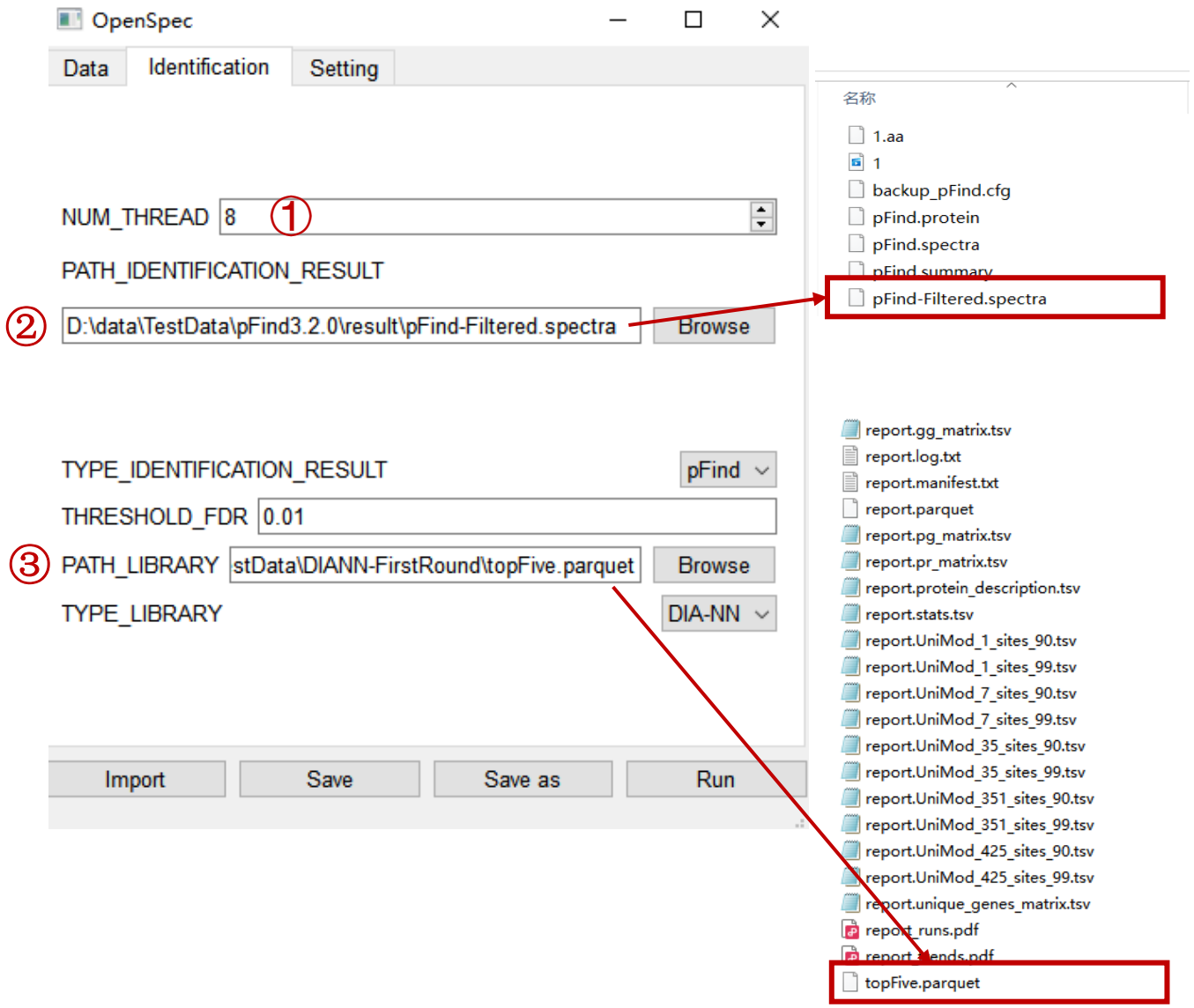
2 Hybrid Spectral Library Construction



① Set **TYPE_FLOW** as **Hybrid-lib**.

② Fill the files into the **PATH_MGF_BIN**.

2 Hybrid Spectral Library Construction

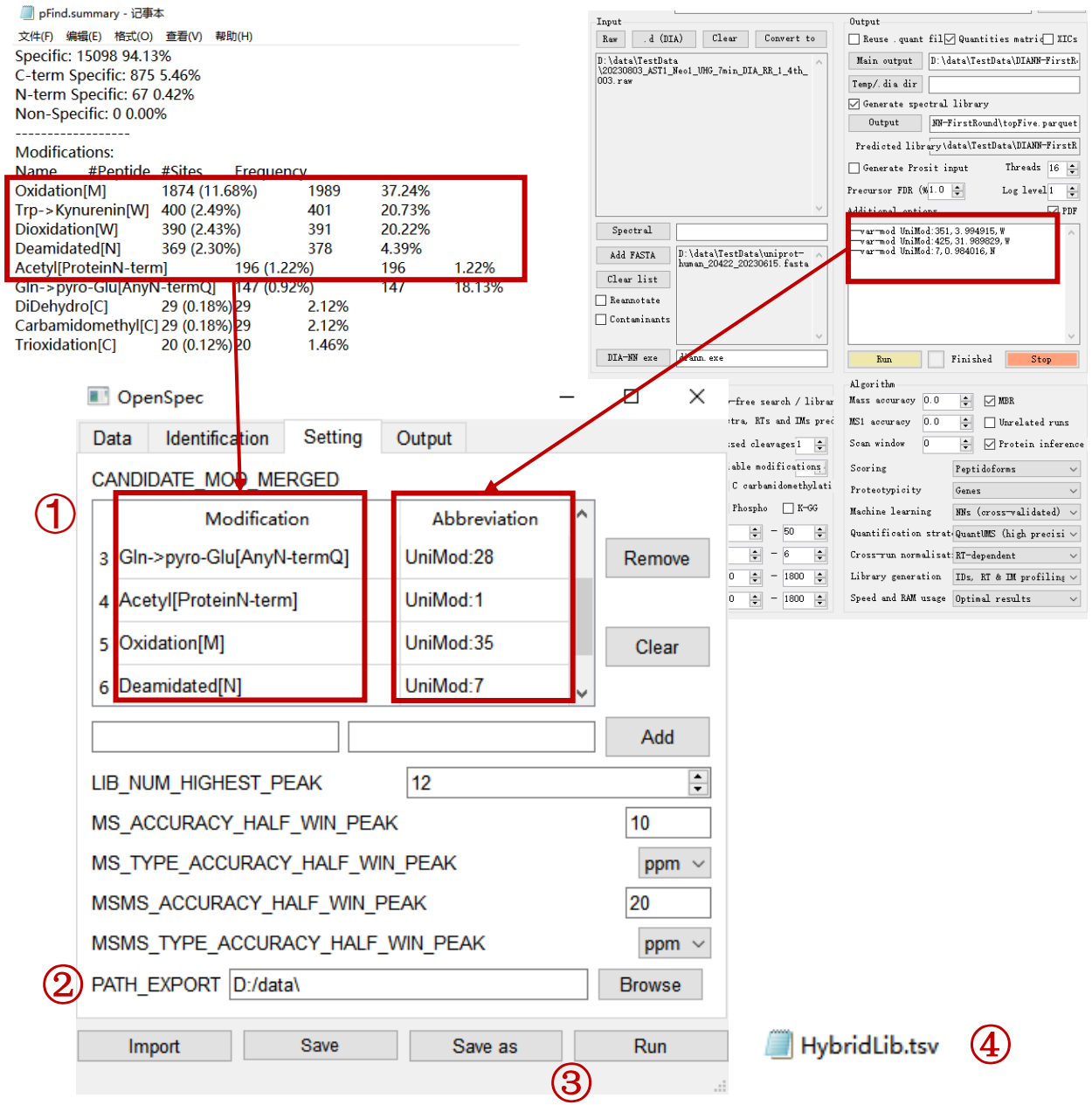


① Adjust **NUM_THREAD** according to your computer's performance; the default is 8.

② Put the identification result file **pFind-Filtered.spectra from pFind** here.

③ Put the **spectral library from first-round search with DIA-NN** here.

2 Hybrid Spectral Library Construction



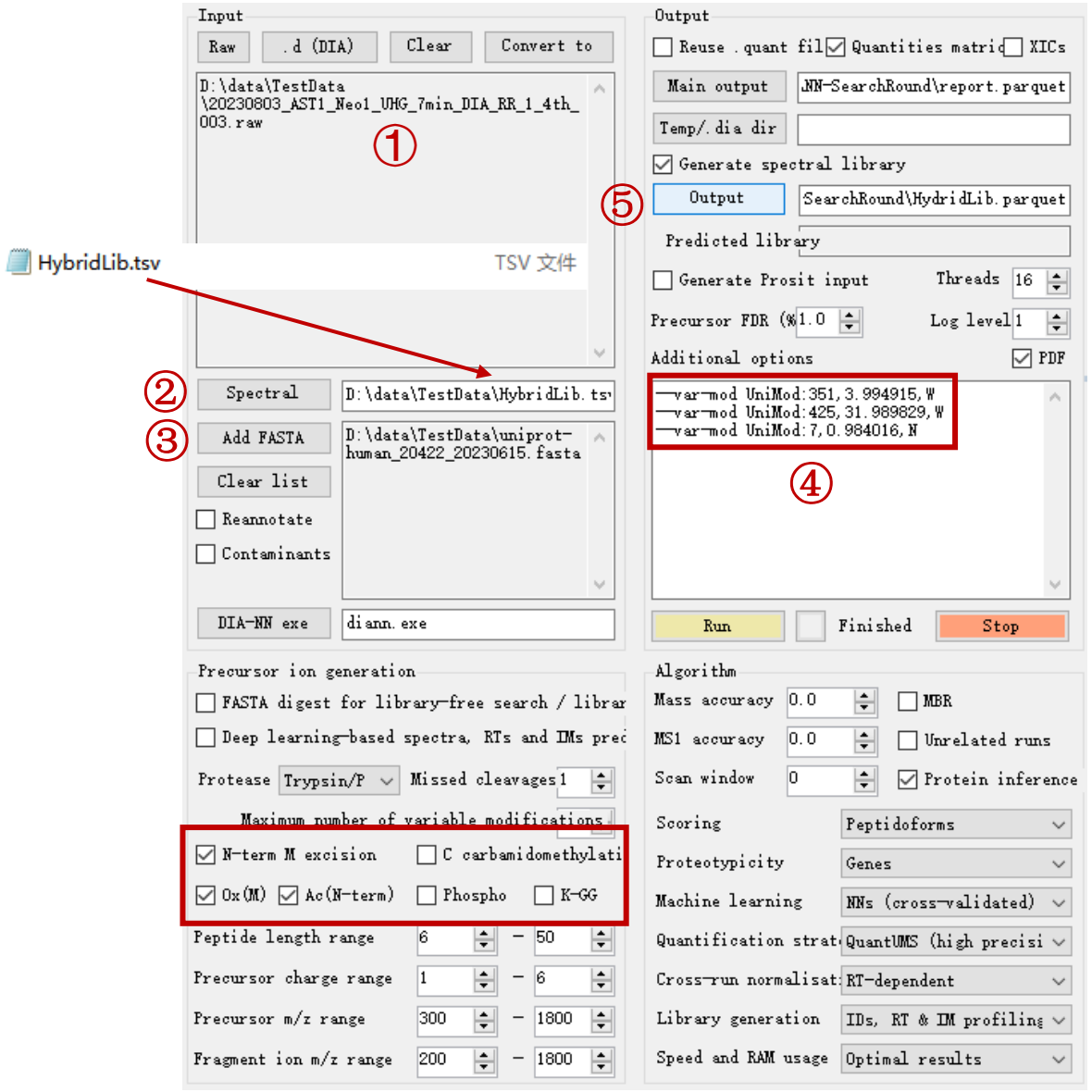
① Set the high-abundance modifications. The **"Modification" column** contains the original modification names from the pFind, while the **"Abbreviation" column** contains the modified names, which should be consistent with the first-round search with DIA-NN.

② Set the **PATH_EXPORT** for saving the results.

③ Click **Save as** button to save the config file. Click **Run** button to start, the progress information will be shown in the command-line interface.

④ The result file.

3 Second-Round Search with DIA-NN.



① Load raw file.

② Set the hybrid spectral library.

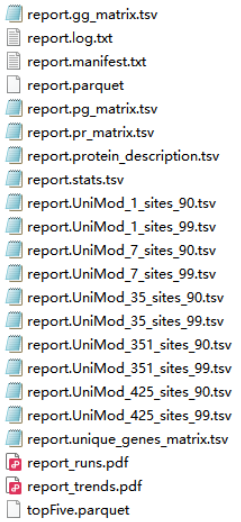
③ Set the fasta database.

④ Set the **high-abundance modifications,** which should be consistent with the first-round search with DIA-NN.

⑤ Select output path.

⑥ The final result.

⑥



Thanks!