

# MSCohort Manual



Version. 202405

### ### Computer configuration

**CPU:** Intel or AMD processor with 64-bit support; 2.3 GHz or faster processor with at least 2 cores is recommended

**RAM:** 16G or higher is recommended

**ROM:** for one raw data (1G) 2G or higher is recommended

**OS:** Windows 10 (x64) or Windows 11 (x64)

**Other:** At present, Spectronaut identification and quantification results can be used for quality analysis by MSCohort.



### ### Demo dataset

The demo dataset has been deposited to the ProteomeXchange Consortium (<https://proteomecentral.proteomexchange.org>) via the iProX partner repository with the dataset identifier PXD050389 (in ProteomeXchange) and IPX0008331000 (in iProX).

The demo datasets available for MSCohort analysis. You can download this dataset for testing and using MSCohort.

This dataset contains raw files of 7 human urine QC DIA data from Orbitrap Exploris 480, Spectronaut analysis results and MSCohort report results.



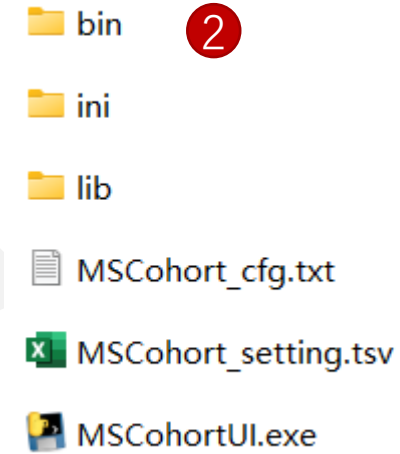
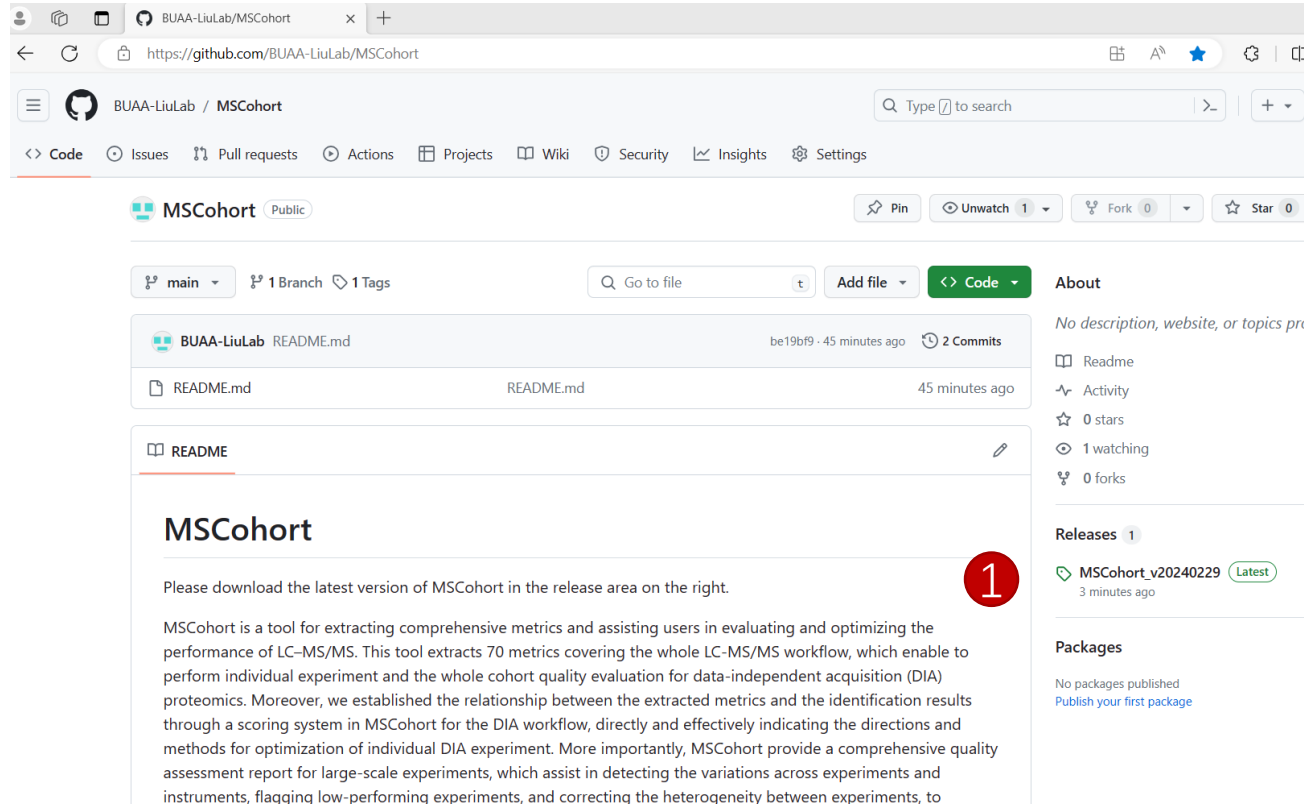
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# **MSCohort download**



# 1. MSCohort download



## ① Login

<https://github.com/BUAA-LiuLab/MS Cohort>

and download the latest version at the right of the page.

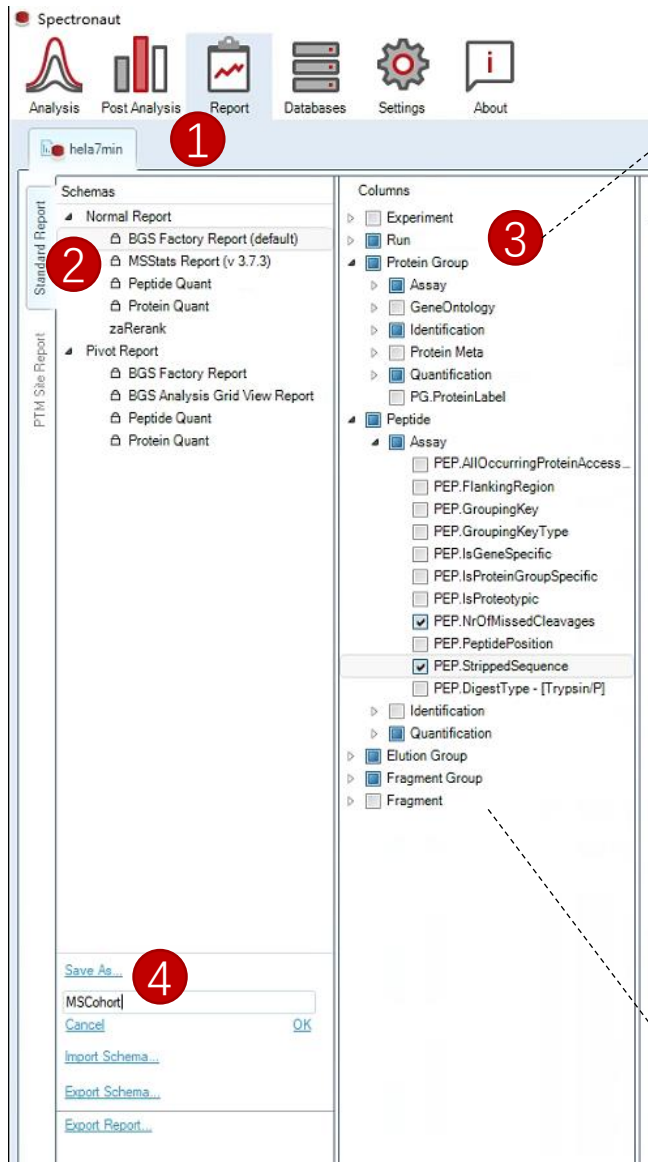
## ② The unzipped MSCohort file.

# **Spectronaut customized report for MSCohort**



# 1. Spectronaut customized report

The columns of information that MSCohort needs to read



**Columns**

- Experiment
- Run
- Protein Group
  - Assay
  - GeneOntology
  - Identification
  - Protein Meta
  - Quantification
  - PG.ProteinLabel
- Peptide
  - Assay
    - PEP.AllOccurringProteinAccess...
    - PEP.FlankingRegion
    - PEP.GroupingKey
    - PEP.GroupingKeyType
    - PEP.IsGeneSpecific
    - PEP.IsProteinGroupSpecific
    - PEP.IsProteotypic
    - ☒ PEP.NrOfMissedCleavages
    - PEP.PeptidePosition
    - ☒ PEP.StrippedSequence
    - PEP.DigestType - [Trypsin/P]
  - Identification
  - Quantification
- Elution Group
- Fragment Group
- Fragment

**Run**

- R.Run Date
- R.Gradient Length [min]
- R.FileName

**Protein Group**

- PG.ProteinGroups
- PG.Qvalue
- PG.Quantity

**Peptide**

- PEP.NrOfMissedCleavages
- PEP.StrippedSequence
- PEP.Quantity

**Elution Group**

- EG.IsDecoy
- EG.ModifiedPeptide
- EG.PrecursorId
- EG.Qvalue
- EG.ApexRT
- EG.DatapointsPerPeak
- EG.DatapointsPerPeak (MS1)
- EG.DeltaRT
- EG.EndRT
- EG.FWHM
- EG.PeakWidth
- EG.StartRT
- EG.SignalToNoise
- EG.TotalQuantity (Settings)

**Fragment Group**

- FG.Charge
- FG.PrecMz
- FG.PrecWindow
- FG.PrecWindowNumber
- FG.CalibratedMassAccuracy (PPM)
- FG.RawMassAccuracy (PPM)

**Fragment**

- F.RawMassAccuracy (PPM)
- F.CalibratedMassAccuracy (PPM)

- ① Choose **Report** Perspective in Spectronaut;
- ② Choose a **Normal Report** format schema as a base to build MSCohort report;
- ③ Choose the **columns** of information that MSCohort needs to read;
- ④ Save the selected columns as a new schema, and name the new report schema as “**MSCohort**”.



## 2. Export MSCohort report from Spectronaut

The screenshot displays the Spectronaut software interface. On the left, the 'Schemas' panel shows a tree view of report types. The 'Normal Report' section is expanded, and the 'MSCohort' option is selected. Below this, the 'Columns' panel shows a list of columns that can be included in the report. The 'Filters' panel shows a list of filters that can be applied. The main area displays a 'Fragment Report [Preview] [Preview]' table with columns: R.Run Date, R.Gradient, R.FileName, PG.ProteinGroup, PG.Qvalue, PG.Quantity, and PEP.NrC. The table contains multiple rows of data, including peptide sequences and their corresponding scores.

1 Choose the MSCohort schema;

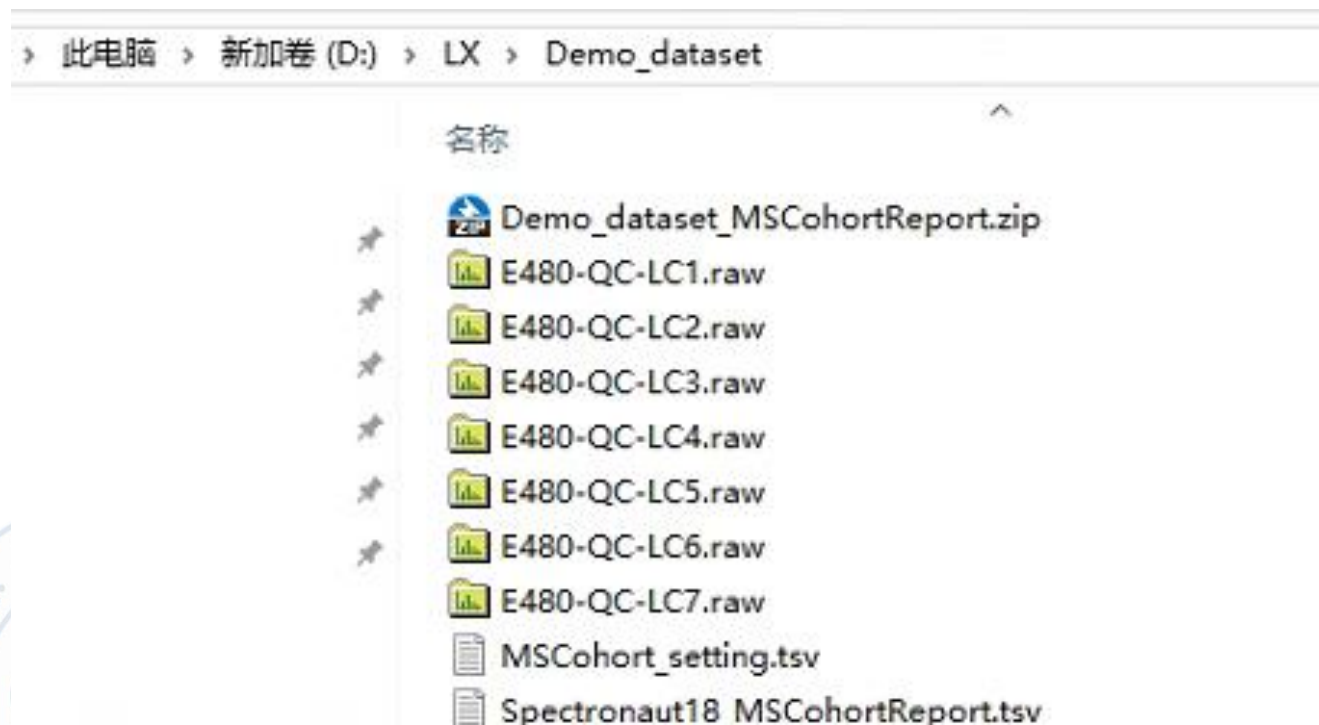
2 Report preview: A preview of how MSCohort report will look like;

3 Export the matrix by clicking on "Export Report..." in the bottom left corner.

# **MSCohort manual for intra- experiment analysis**



# Demo dataset

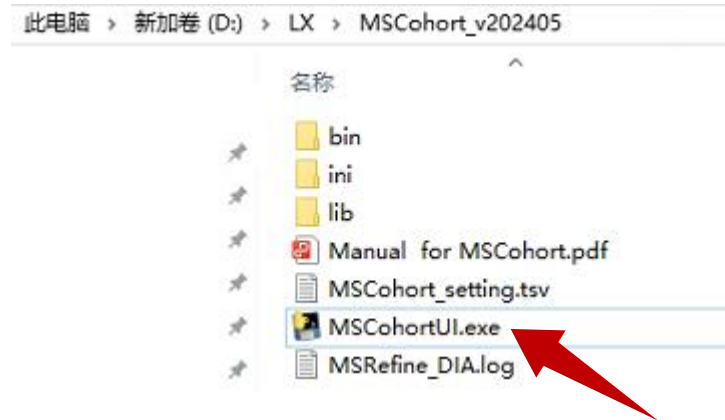


Taking the E480-QC-LC1.raw file as an example to demonstrate the workflow of intra-experiment analysis.

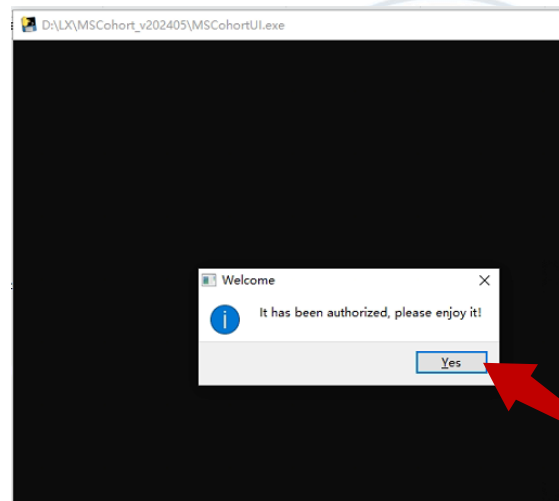
Intra-experiment analysis enables the systematic evaluation and optimization of individual DIA experiments.

# 1. Analyzing with MSCohort

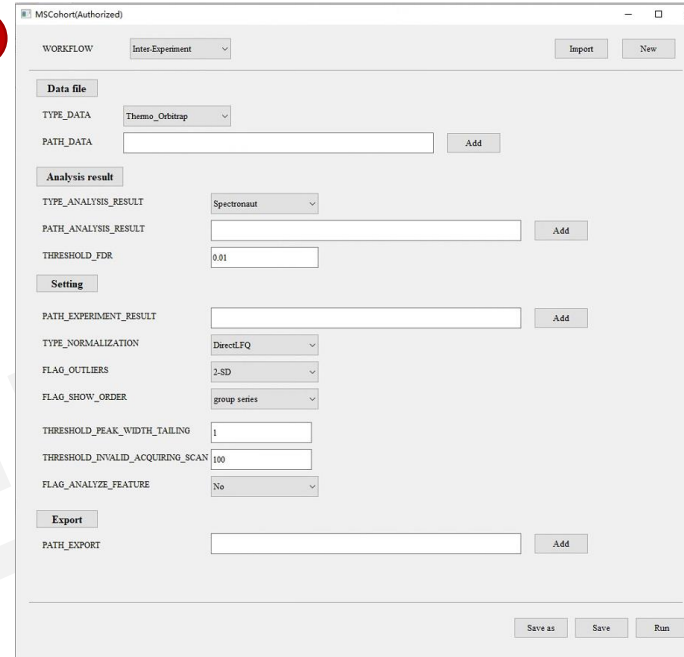
1



2



3



- ① Double-click **MSCohortUI.exe**;
- ② Click **Yes** ;
- ③ The MSCohortUI.exe settings screen is displayed.

# 1. Analyzing with MSCohort

The screenshot shows the MSCohort(Authorized) software interface. It has a light gray background with various input fields and buttons. The interface is organized into sections: WORKFLOW, Data file, TYPE\_DATA, PATH\_DATA, Analysis result, Setting, and Export. Red circles with numbers 1 through 5 are placed over specific elements: 1 is over the WORKFLOW dropdown menu, 2 is over the TYPE\_DATA dropdown menu, 3 is over the PATH\_DATA text input field, 4 is over the PATH\_ANALYSIS\_RESULT text input field, and 5 is over the PATH\_EXPORT text input field. The PATH\_DATA field has an 'Add' button next to it. The PATH\_ANALYSIS\_RESULT field also has an 'Add' button. The PATH\_EXPORT field has an 'Add' button. At the bottom right, there are 'Save as', 'Save', and 'Run' buttons.

1 WORKFLOW Intra-Experiment Inter-Experiment Inter-Experiment Intra-Experiment Import New

2 TYPE\_DATA Thermo\_Orbitrap Thermo\_Orbitrap Thermo\_Orbitrap Bruker\_timsTOF SCIEX\_ZenoTOF

3 PATH\_DATA D:/LX/Demo\_dataset/E480-QC Add

4 PATH\_ANALYSIS\_RESULT Spectronaut D:/LX/Demo\_dataset/Spectronaut18\_MSCohortReport.tsv Add

5 PATH\_EXPORT D:/LX/Demo\_dataset Add

THRESHOLD\_FDR 0.01

THRESHOLD\_PEAK\_WIDTH\_TAILING 1

THRESHOLD\_INVALID\_ACQUIRING\_SCAN 100

FLAG\_ANALYZE\_FEATURE No

Save as Save Run

- ① Select **WORKFLOW** as **Intra-experiment**;
- ② Select **TYPE\_DATA** according to the data type ;
- ③ Click **Add** to select the raw file into the **PATH\_DATA**;
- ④ Click **Add** to select the Spectronaut customized report for MSCohort into the **PATH\_ANALYSIS\_RESULT**;
- ⑤ Click **Add** to set the **PATH\_EXPORT** for saving the results.

## Note:

Space (“ ”) cannot exist in the file directory (including PATH\_DATA, PATH\_ANALYSIS\_RESULT, and PATH\_EXPORT ), which will affect the normal running of the program.

# 1. Analyzing with MSCohort

The screenshot shows the MSCohort(Authorized) window. It has a top bar with 'Import' and 'New' buttons. Below is a 'WORKFLOW' section with a dropdown set to 'Intra-Experiment'. The main area is divided into four sections: 'Data file', 'Analysis result', 'Setting', and 'Export'. The 'Data file' section has 'TYPE\_DATA' set to 'Thermo\_Orbitrap' and 'PATH\_DATA' set to 'D:/LX/Demo\_dataset/E480-QC-LC1.raw'. The 'Analysis result' section has 'TYPE\_ANALYSIS\_RESULT' set to 'Spectronaut', 'PATH\_ANALYSIS\_RESULT' set to 'D:/LX/Demo\_dataset/Spectronaut1\$ MSCohortReport.tsv', and 'THRESHOLD\_FDR' set to '0.01'. The 'Setting' section has 'THRESHOLD\_PEAK\_WIDTH\_TAILING' set to '1', 'THRESHOLD\_INVALID\_ACQUIRING\_SCAN' set to '100', and 'FLAG\_ANALYZE\_FEATURE' set to 'Yes'. The 'Export' section has 'PATH\_EXPORT' set to 'D:/LX/Demo\_dataset'. At the bottom, there are 'Save as', 'Save', and 'Run' buttons. Red circles with numbers 1 through 5 are placed over the following elements: 1. 'THRESHOLD\_PEAK\_WIDTH\_TAILING' input field; 2. 'THRESHOLD\_INVALID\_ACQUIRING\_SCAN' input field; 3. 'FLAG\_ANALYZE\_FEATURE' dropdown menu; 4. 'Save as' button; 5. 'Run' button.

WORKFLOW: Intra-Experiment

Data file

TYPE\_DATA: Thermo\_Orbitrap

PATH\_DATA: D:/LX/Demo\_dataset/E480-QC-LC1.raw

Add

Analysis result

TYPE\_ANALYSIS\_RESULT: Spectronaut

PATH\_ANALYSIS\_RESULT: D:/LX/Demo\_dataset/Spectronaut1\$ MSCohortReport.tsv

Add

THRESHOLD\_FDR: 0.01

Setting

1 THRESHOLD\_PEAK\_WIDTH\_TAILING: 1

2 THRESHOLD\_INVALID\_ACQUIRING\_SCAN: 100

3 FLAG\_ANALYZE\_FEATURE: Yes

Export

PATH\_EXPORT: D:/LX/Demo\_dataset

Add

4 Save as

5 Save Run

- ① **THRESHOLD\_PEAK\_WIDTH\_TAIL** (default setting 1, user adjustable parameter according to experimental condition), set as 1 represents that precursors with peak width more than 1 minutes are used to calculate the proportion of precursors with long eluting width;
- ② **THRESHOLD\_INVALID\_ACQUIRING** (default setting 100, user adjustable parameter according to experimental condition), set as 100 represents that the retention time length from the start to the number of identified MS2 scans per minute more than 100 as chromatographic invalid acquiring time;
- ③ **FLAG\_ANALYZE\_FEATURE** (default setting No). This analysis may take a long time for timsTOF and zenoTOF data, it is recommended to set as No for for timsTOF and zenoTOF data;
- ④ Click **Save as** button to save the config file;
- ⑤ Click **Run** button to start the MSCohort, the progress information will be shown in the command-line interface.

# 1. Analyzing with MSCohort

The screenshot shows the MSCohort software interface. At the top, there is a 'WORKFLOW' dropdown set to 'Intra-Experiment' and buttons for 'Import' and 'New'. A red circle with the number '2' is placed over the 'New' button. Below this, there are three main sections: 'Data file', 'Analysis result', and 'Setting'. The 'Data file' section has a 'TYPE\_DATA' dropdown set to 'Thermo\_Orbitrap' and a 'PATH\_DATA' text field containing 'D:/LX/Demo\_dataset/E480-QC-LC1.raw', with an 'Add' button next to it. The 'Analysis result' section has a 'TYPE\_ANALYSIS\_RESULT' dropdown set to 'Spectronaut', a 'PATH\_ANALYSIS\_RESULT' text field containing 'D:/LX/Demo\_dataset/Spectronaut18\_MSCohortReport.tsv', and a 'THRESHOLD\_FDR' text field containing '0.01', each with an 'Add' button. The 'Setting' section has three text fields: 'THRESHOLD\_PEAK\_WIDTH\_TAILING' (1), 'THRESHOLD\_INVALID\_ACQUIRING\_SCAN' (100), and a 'FLAG\_ANALYZE\_FEATURE' dropdown set to 'Yes'. At the bottom, there is an 'Export' section with a 'PATH\_EXPORT' text field containing 'D:/LX/Demo\_dataset' and an 'Add' button. A red circle with the number '1' is placed over the 'PATH\_EXPORT' text field. At the very bottom of the window are buttons for 'Save as', 'Save', and 'Run'.

## Note:

① **PATH\_EXPORT** : The output MSCohort analysis results will be stored in a new folder under the PATH\_EXPORT directory, named **MSCohort\_“filename”** folder.

**Do not save the same PATH\_DATA under a same folder, the results will be **overwritten**.**

② Choose **New** for a new experiments

③ Check the filename in **PATH\_DATA** must in the Spectronaut report in **PATH\_ANALYSIS\_RESULT**.



## 2. MSCohort Results

1

> LX > Demo\_dataset > MSCohort\_E480-QC-LC1

名称	修改日期
picture	2024/
Analysis_Report.html	2024/
INFO_Summary.txt	2024/
INFO_Chromatography.txt	2024/
INFO_Cycle_MS1.txt	2024/
INFO_Cycle_MS2.txt	2024/
INFO_Feature.txt	2024/
INFO_ID.txt	2024/
INFO_Mass_Deviation.txt	2024/
INFO_MS1.txt	2024/
INFO_MS1_PEAKS.txt	2024/
INFO_MS2.txt	2024/
INFO_MS2_PEAKS.txt	2024/
INFO_peptides.txt	2024/
INFO_Scans.txt	2024/

1.3 Metric-Score Analysis

2

① The MSCohort results ;

② Double clicking [Analysis\\_Report.html](#), the report will be preformed in the browser.

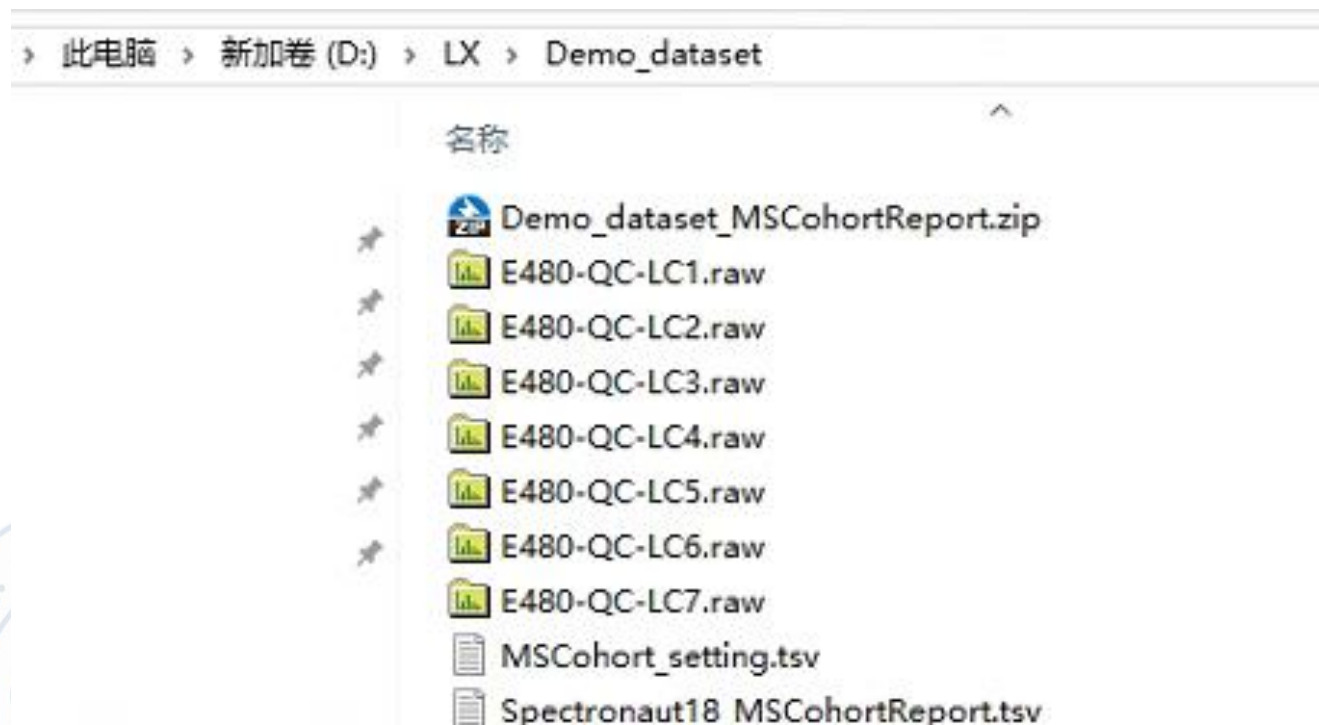




# **MSCohort manual for inter- experiment analysis**



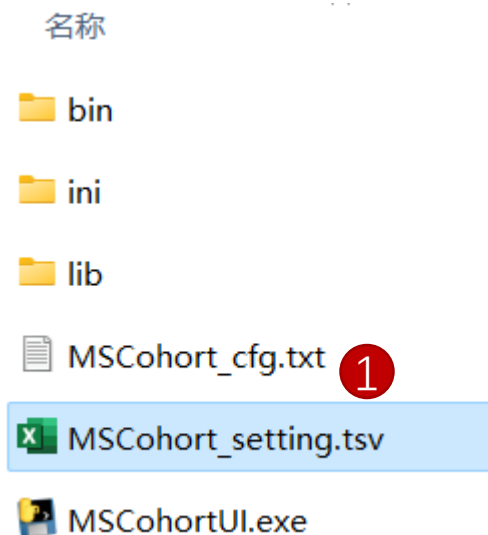
# Demo dataset



Taking the 7 E480-QC raw files as an example to demonstrate the workflow of inter-experiment analysis.

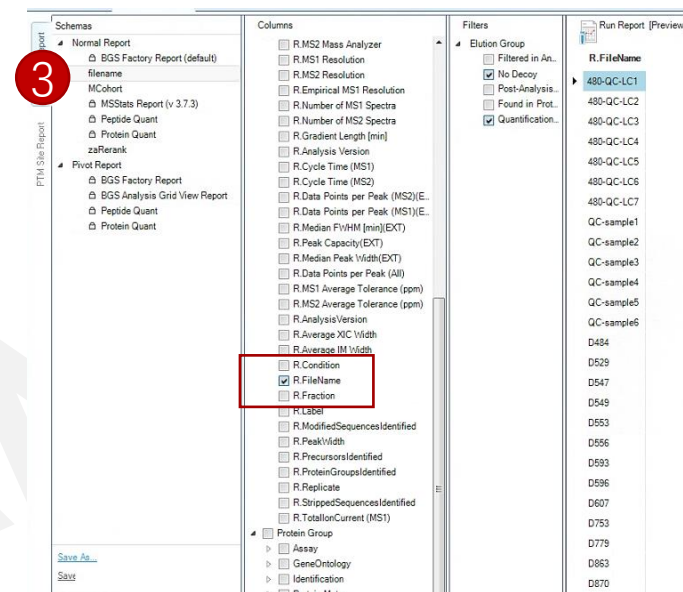
Inter-experiment analysis enables the systematic quality evaluation and low-quality experiments detection for multiple DIA experiments.

# 1. Preparation for MSCohort\_setting.tsv file



2

Group Name	Raw Name	Experiment	Threshold
E480-QC	E480-QC-LC1	E480-QC1	10
E480-QC	E480-QC-LC2	E480-QC2	10
E480-QC	E480-QC-LC3	E480-QC3	10
E480-QC	E480-QC-LC4	E480-QC4	10
E480-QC	E480-QC-LC5	E480-QC5	10
E480-QC	E480-QC-LC6	E480-QC6	10
E480-QC	E480-QC-LC7	E480-QC7	10



- ① Double clicking [MSCohort\\_setting.tsv](#);
- ② Fill the columns as the example file showed:
  - The first column is the Group Name.
  - The second column is the Raw Name, which is the same as [R.FileName](#) reported from Spectronaut.
  - The third column is the Raw Name for short, which is convenient for display in large MSCohort report. This column could also be the same as Raw Name column.
  - The fourth column is the intensity threshold, protein/peptide intensity that less than this threshold would be replaced as NaN, and would not be used for subsequent Pearson correlation analysis.
- ③ For large-scale cohorts, users can get the Raw Name list from Spectronaut by exporting only [R.FileName](#) column.

## 2. Analyzing with MSCohort

The screenshot shows the MSCohort software interface with the following configuration:

- Workflow:** Inter-Experiment
- Data file:**
  - TYPE\_DATA:** Thermo\_Orbitrap
  - PATH\_DATA:** (empty)
- Analysis result:**
  - TYPE\_ANALYSIS\_RESULT:** Spectronaut
  - PATH\_ANALYSIS\_RESULT:** D:/LX/Demo\_dataset/Spectronaut18\_MSCohortReport.tsv
  - THRESHOLD\_FDR:** 0.01
- Setting:**
  - PATH\_EXPERIMENT\_RESULT:** D:/LX/Demo\_dataset/MSCohort\_setting.tsv
  - TYPE\_NORMALIZATION:** DirectLFQ
  - FLAG\_OUTLIERS:** 2-SD
  - FLAG\_SHOW\_ORDER:** group series
  - THRESHOLD\_PEAK\_WIDTH\_TAILING:** 1
  - THRESHOLD\_INVALID\_ACQUIRING\_SCAN:** 100
  - FLAG\_ANALYZE\_FEATURE:** No
- Export:**
  - PATH\_EXPORT:** D:/LX/Demo\_dataset

Buttons at the bottom: Save as, Save, Run.

- ① Set **WORKFLOW** as **Inter-experiment**;
- ② Select **TYPE\_DATA** according to the data type ;
- ③ The **PATH\_DATA** could be empty. MSCohort support the inter-experimental analysis mainly based on Spectronaut result, eliminating the need to submit raw files, which will obtain the result in a relatively **short time**. It is recommended for large cohort analysis.

In addition, users could choose to add the raw data. MSCohort would provide comprehensive analysis reports not only for inter-experiment, but also for intra-experiment analysis. This may **take a long time**.

- ④ Click **Add** to select the Spectronaut customized report for MSCohort into the **PATH\_ANALYSIS\_RESULT**;
- ⑤ Click **Add** to select the **MSCohort\_setting.tsv** into the **PATH\_EXPERIMENT\_RESULT**;

## 2. Analyzing with MSCohort

The screenshot shows the MSCohort software interface with the following configuration:

- Workflow:** Inter-Experiment
- Data file:**
  - TYPE\_DATA: Thermo\_Orbitrap
  - PATH\_DATA: (empty)
- Analysis result:**
  - TYPE\_ANALYSIS\_RESULT: Spectronaut
  - PATH\_ANALYSIS\_RESULT: D:/LX/Demo\_dataset/Spectronaut18\_MSCohortReport.tsv
  - THRESHOLD\_FDR: 0.01
- Setting:**
  - PATH\_EXPERIMENT\_RESULT: D:/LX/Demo\_dataset/MSCohort\_setting.tsv
  - TYPE\_NORMALIZATION: DirectLFQ
  - FLAG\_OUTLIERS: 2-SD
  - FLAG\_SHOW\_ORDER: group series
  - THRESHOLD\_PEAK\_WIDTH\_TAILING: 1
  - THRESHOLD\_INVALID\_ACQUIRING\_SCAN: 100
  - FLAG\_ANALYZE\_FEATURE: No
- Export:**
  - PATH\_EXPORT: D:/LX/Demo\_dataset

Buttons at the bottom: Save as, Save, Run.

- ① Choose normalization strategies in **TYPE\_NORMALIZATION**. MSCohort support directLFQ, MaxLFQ, and Quantile normalization (default is directLFQ);
- ② Choose **FLAG\_OUTLIERS threshold** according to the experiment condition (default is 2\*SD, users could adjust the threshold (Notes for modifying the scoring criteria));
- ③ Choose **FLAG\_SHOW\_ORDER**. Group series represents the experiment order showed in MSCohort report is the same as the **MSCohort\_setting.tsv; time series** represents the experiment order showed in MSCohort report is sorted by run date.
- ④ Set **THRESHOLD\_PEAK\_WIDTH\_TAIL**, **THRESHOLD INVALID\_ACQUIRING**, **FLAG\_ANALYZE\_FEATURE** as intra-experiment analysis?

## 2. Analyzing with MSCohort

The screenshot shows the MSCohort(Authorized) window. It has a top bar with 'WORKFLOW' set to 'Inter-Experiment' and buttons for 'Import' and 'New'. Below this are four main sections: 'Data file', 'Analysis result', 'Setting', and 'Export'. The 'Data file' section has 'TYPE\_DATA' set to 'Thermo\_Orbitrap' and an empty 'PATH\_DATA' field with an 'Add' button. The 'Analysis result' section has 'TYPE\_ANALYSIS\_RESULT' set to 'Spectronaut', 'PATH\_ANALYSIS\_RESULT' set to 'D:/LX/Demo\_dataset/Spectronaut1\$ \_MSCohortReport.tsv', and 'THRESHOLD\_FDR' set to '0.01'. The 'Setting' section has 'PATH\_EXPERIMENT\_RESULT' set to 'D:/LX/Demo\_dataset/MSCohort\_setting.tsv', 'TYPE\_NORMALIZATION' set to 'DirectLFQ', 'FLAG\_OUTLIERS' set to '2-SD', 'FLAG\_SHOW\_ORDER' set to 'group series', 'THRESHOLD\_PEAK\_WIDTH\_TAILING' set to '1', 'THRESHOLD\_INVALID\_ACQUIRING\_SCAN' set to '100', and 'FLAG\_ANALYZE\_FEATURE' set to 'No'. The 'Export' section has 'PATH\_EXPORT' set to 'D:/LX/Demo\_dataset'. At the bottom right, there are three buttons: 'Save as' (annotated with a red circle 2), 'Save' (annotated with a red circle 2), and 'Run' (annotated with a red circle 3). A red circle 1 is placed next to the 'PATH\_EXPORT' field in the 'Export' section.

WORKFLOW: Inter-Experiment [Import] [New]

**Data file**

TYPE\_DATA: Thermo\_Orbitrap

PATH\_DATA: [Add]

**Analysis result**

TYPE\_ANALYSIS\_RESULT: Spectronaut

PATH\_ANALYSIS\_RESULT: D:/LX/Demo\_dataset/Spectronaut1\$ \_MSCohortReport.tsv [Add]

THRESHOLD\_FDR: 0.01

**Setting**

PATH\_EXPERIMENT\_RESULT: D:/LX/Demo\_dataset/MSCohort\_setting.tsv [Add]

TYPE\_NORMALIZATION: DirectLFQ

FLAG\_OUTLIERS: 2-SD

FLAG\_SHOW\_ORDER: group series

THRESHOLD\_PEAK\_WIDTH\_TAILING: 1

THRESHOLD\_INVALID\_ACQUIRING\_SCAN: 100

FLAG\_ANALYZE\_FEATURE: No

**Export**

PATH\_EXPORT: D:/LX/Demo\_dataset [Add]

[Save as] [Save] [Run]

- ① Set the **PATH\_EXPORT** for saving the results. The output MSCohort analysis results will be stored in a new folder under the PATH\_EXPORT directory, named MSCohort \_“year+month+day” folder.

**Do not save the different experiments results under a same folder, the results will be **overwritten**.**

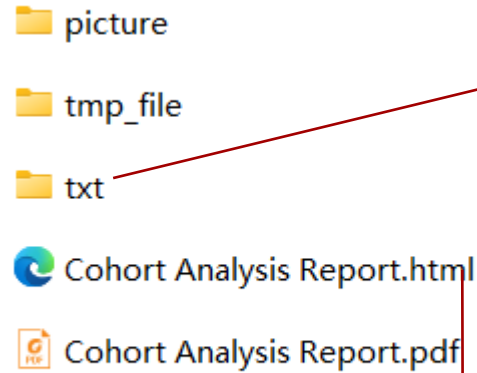
- ② Click **Save as** button to save the config file;
- ③ Click **Run** button to start the MSCohort, the progress information will be shown in the command-line interface.

### Note:

Space (“ ”) cannot exist in the file directory (including PATH\_DATA, PATH\_ANALYSIS\_RESULT, PATH\_EXPERIMENT\_RESULT, and PATH\_EXPORT ), which will affect the normal running of the program.

# 3. MSCohort Results

1



3

- INFO\_Pro0\_MissingValue\_Count.txt
- INFO\_Pro1\_Intensity.txt
- INFO\_Pro2\_Origin\_Intensity.txt
- INFO\_Pro3\_Coefficient\_Var.txt
- INFO0\_Experiment\_Outlier\_Score.txt
- INFO0\_Identification\_Count.txt
- INFO0\_Inter\_Experiment\_Scores.txt
- INFO0\_Inter\_Experiment\_values.txt
- INFO0\_Intra\_Experiment\_Scores.txt
- INFO0\_Intra\_Experiment\_values.txt
- INFO1\_Pro0\_MissingValue\_Count.txt
- INFO2\_Pro1\_Intensity.txt
- INFO3\_Pro2\_Origin\_Intensity.txt
- INFO4\_Pro3\_Coefficient\_Var.txt
- INFO5\_Pre\_RetentionTime.txt

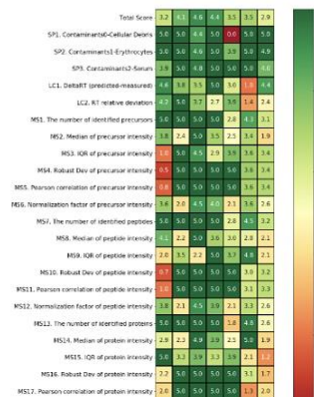
- ① The MSCohort results ;
- ② Double clicking **Cohort Analysis Report.html**, the report will be showed in the browser.
- ③ Double clicking **txt** folder, the outputs are also exported to simple tab-delimited text files.

2

## 1. Overview of Dataset

### 1.1 Score of Inter-experiment Metrics

Inter-experiment metrics are computed across multiple experiments to assess the quality for the whole cohort quality data.



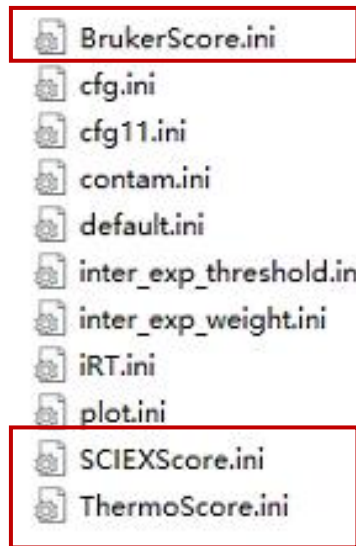
# **Notes for modifying the scoring criteria**





# 1. Modifying the scoring criteria for intra-experiment analysis

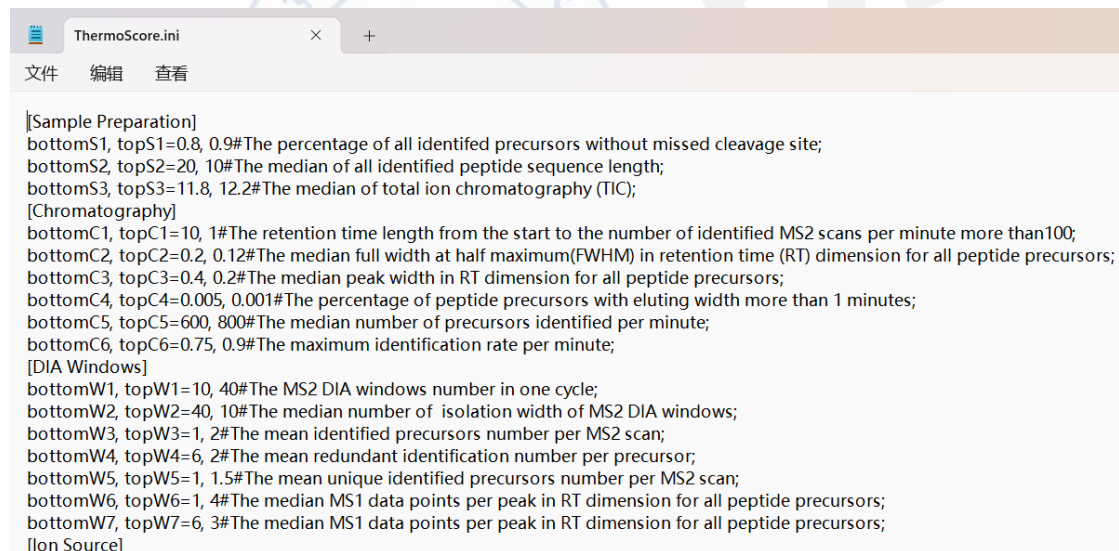
1



① Open the **ini** folder, there will be three parameter files related to intra-experiment scoring: ThermoScore.ini, BrukerScore.ini, SCIEXScore.ini;

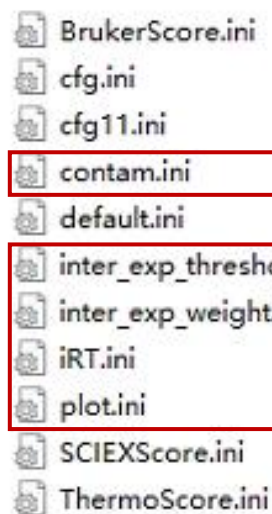
② Click the file to modify the second-level scoring threshold. If you do not modify it, it will be the default value. Closing the file and running the software, it will score according to the standard you set.

2

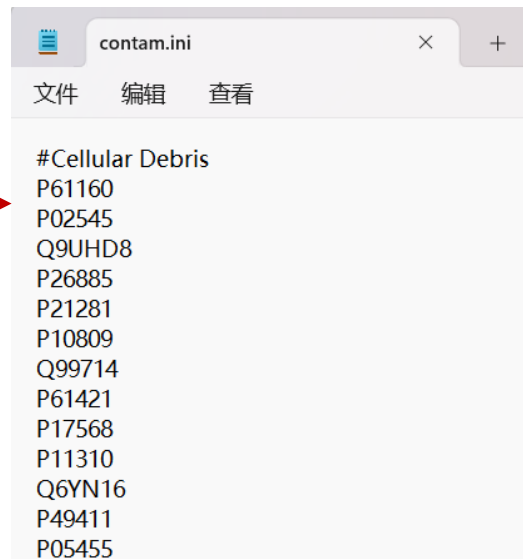


# 1. Modifying the scoring criteria for inter-experiment analysis

1



2

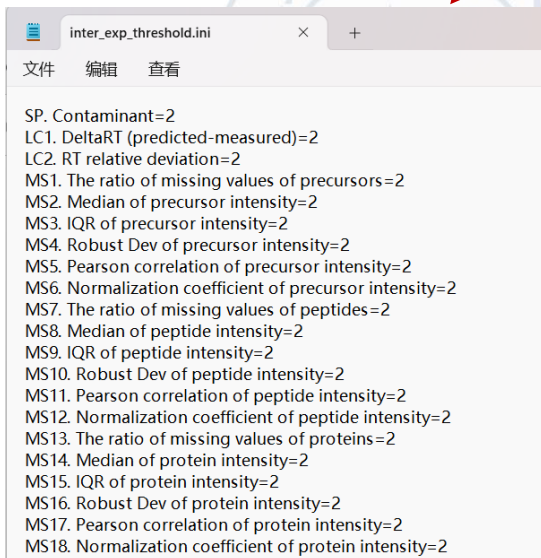


① Open the **ini** folder, there will be five parameter files related to inter-experiment scoring: **contam.ini**, **inter\_exp\_threshold.ini**, **inter\_exp\_weight.ini**, **iRT.ini**, **plot.ini**;

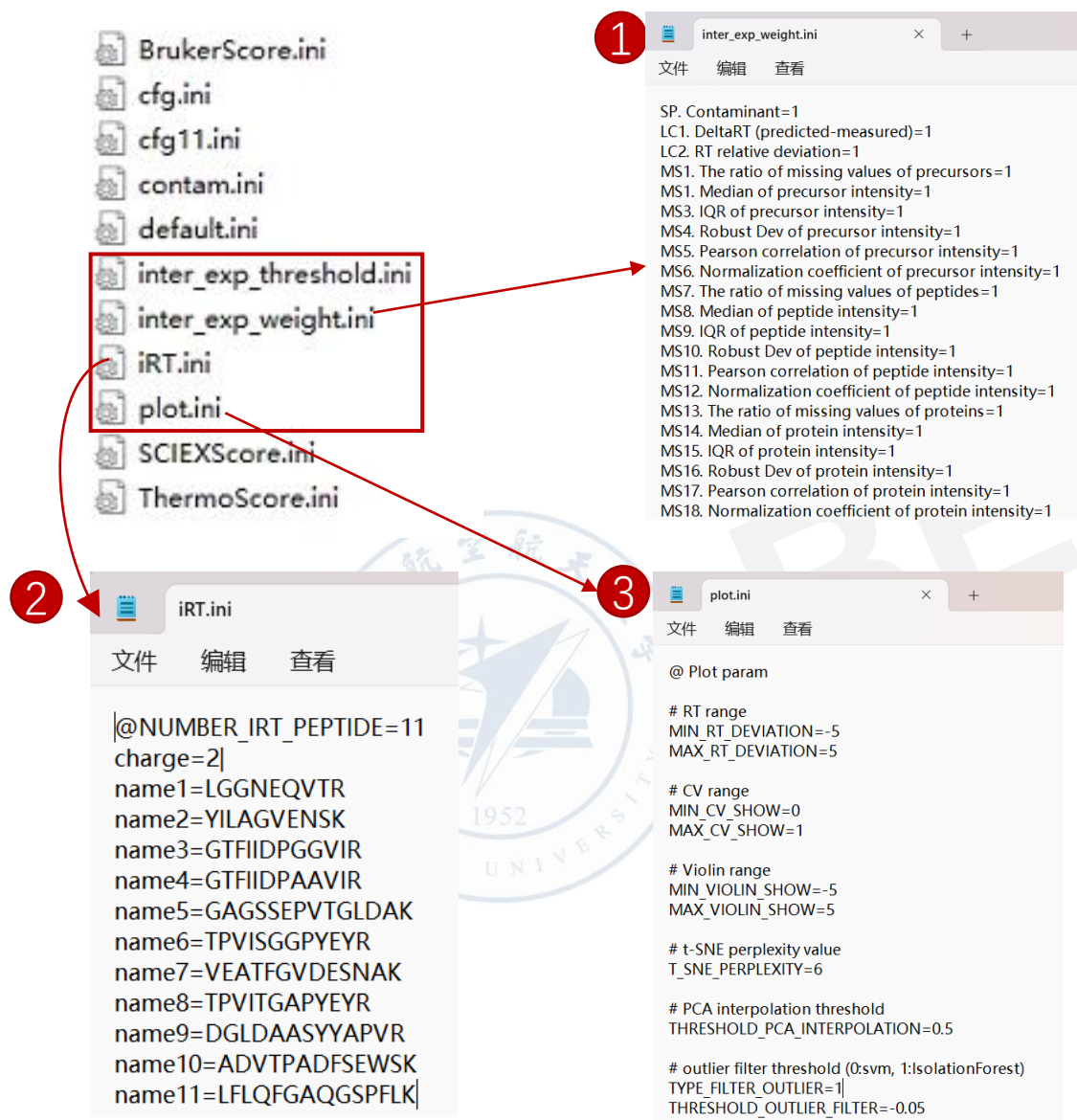
② Open the **contam.ini**, users can modify the list of contaminating proteins, # begins with contaminants category, enter the list of contaminating proteins under this category below.

③ Open the **inter\_exp\_threshold.ini**, users can modify the scoring threshold. For each metric, we initially defined potentially outlier experiments as those with a value more than two standard deviations ( $2 \times SD$ ) from the median. If you do not modify it, it will be the default value as 2. Closing and saving the file, it will score according to the standard you set.

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# 1. Modifying the scoring criteria for inter-experiment analysis



- ① Open the `inter_exp_weight.ini`, users can modify the scoring weight for each metric. We initially defined the weight as 1 for each metric. If you do modify it, closing and saving the file, it will score according to the standard you set.
- ② Open the `iRT.ini`, users can modify the list of iRT peptide sequence. The default iRT peptide sequence is the 11 non-naturally occurring synthetic peptides from the iRT kit (Biognosys). Users can modify the sequence according to experimental conditions.
- ③ Open the `plot.ini`, users can modify the parameters related to plot. RT range is y axis range showed in RT deviation analysis plot; CV range is the y axis range showed in CV plot; Violin range is the y axis range showed in Intensity ratio distribution plot; TYPE\_FILTER\_OUTLIERS is the approach to detect outliers (0: one-class SVM; 1: isolation forest); THRESHOLD\_OUTLIER\_FILTER is threshold to flag as outliers (In the data we tested, the SVM model threshold was set to -1; the isolation forest was set to -0.05).

# Thanks!

