

pGlycoQuant Manual

version.2022.05



Version: pGlycoQuant v202205

Release Date: 2022.05.01

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) 5G or higher is recommended

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

Other: MSFileReader 3.0 Sp1 or higher is needed. If MSFileReader 3.0 has not been installed, please download MSFileReader.3.0.Sp1.zip and install it.

Description

At present, pFind, pGlyco, Byonic and MSFragger software glycosylation identification results can be used for quantification by pGlycoQuant.

Notes for running Byonic result

1. It is found that the name of mass spectrum data recorded by Byonic software is inconsistent with the original data, when running pGlycoQuant in Byonic mode, it should be guaranteed that the name of the mass spectrum data recorded in the Byonic result file is the same as that of the entered mass spectrum data.
2. Byonic glycosylation modification reliable results screening commonly used scores are Score and LogProb, rather than FDR. FDR cannot be modified on the pGlycoQuant interface. To modify B4_THRESHOLD_SCORE_BYONIC and B5_THRESHOLD_PROB_BYONIC in the config file (default: 200 and 2, indicating score \geq 200 and absolute value of LogProb \geq 2).
3. Byonic ini files are required for quantification, in the ./ini/ini_Byonic directory.

Notes for running MSFragger result

MSFragger ini files are required for quantification, in the ./ini/ini_MSFragger directory.

Cite us

Weiqian Cao, et. al. pGlycoQuant with a deep residual network for precise and minuscule-missing-value quantitative glycoproteomics enabling the functional exploration of site-specific glycosylation. bioRxiv 2021.11.15.468561.

doi: <https://doi.org/10.1101/2021.11.15.468561>

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pGlyco & pGlycoQuant

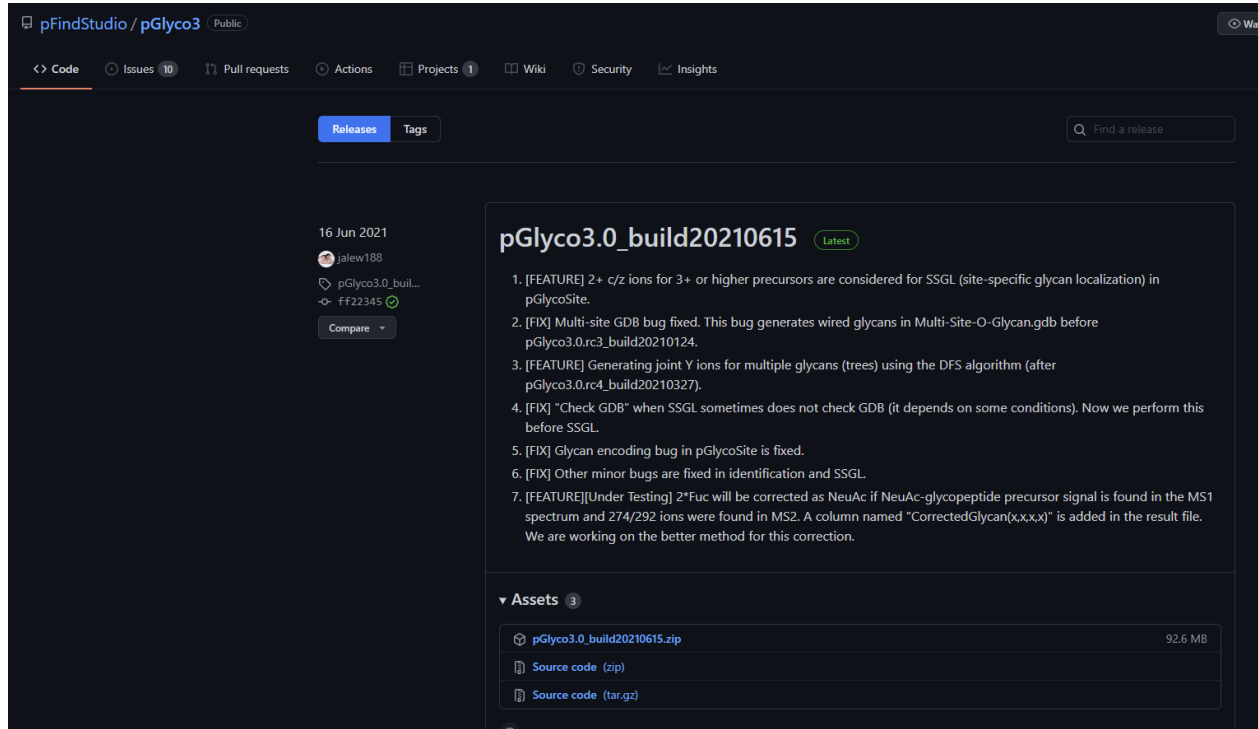
Download and Regist



1 Identification with pGlyco

1.1 pGlyco download

①



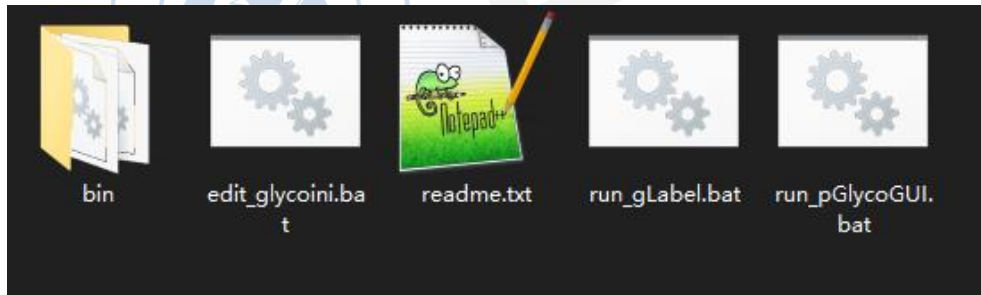
① Login

<https://github.com/pFindStudio/pGlyco3/releases>

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

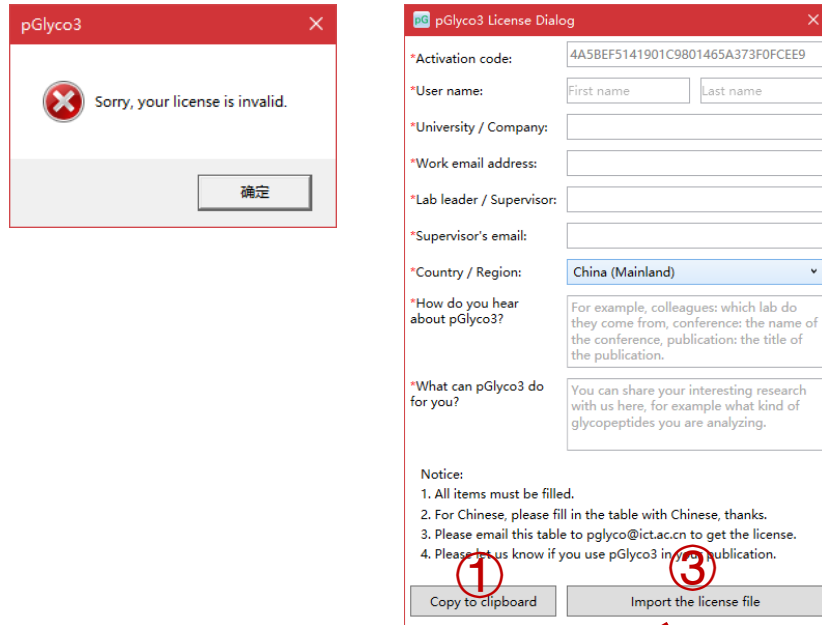
② The unzipped pGlyco files.

②

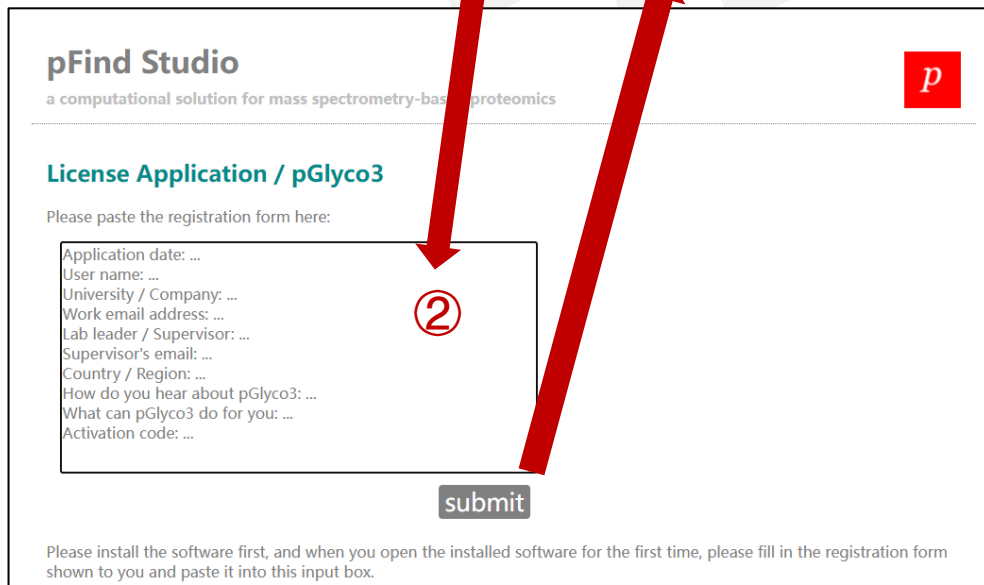


1 Identification with pGlyco

1.2 pGlyco register



The image shows two windows from the pGlyco3 software. On the left is a small error dialog titled 'pGlyco3' with a red 'X' icon and the text 'Sorry, your license is invalid.' with a '确定' (OK) button. On the right is the 'pGlyco3 License Dialog' window. It contains several input fields: 'Activation code' (pre-filled with 4A5BEF5141901C9801465A373F0FCEE9), 'User name' (First name and Last name), 'University / Company', 'Work email address', 'Lab leader / Supervisor', 'Supervisor's email', and 'Country / Region' (set to China (Mainland)). There are also text areas for 'How do you hear about pGlyco3?' and 'What can pGlyco3 do for you?'. At the bottom, there are two buttons: 'Copy to clipboard' (labeled with a red circled 1) and 'Import the license file' (labeled with a red circled 3). A 'Notice' section at the bottom left lists four instructions.



The image shows the 'pFind Studio' web interface. The header includes the 'pFind Studio' logo and the tagline 'a computational solution for mass spectrometry-based proteomics'. Below this is the 'License Application / pGlyco3' section. It instructs the user to 'Please paste the registration form here:'. A large text area contains the registration form fields, which are identical to the ones in the 'pGlyco3 License Dialog' window. A red arrow labeled with a circled 2 points from the 'Copy to clipboard' button in the dialog to this text area. At the bottom of the text area is a 'submit' button. Another red arrow labeled with a circled 3 points from the 'Import the license file' button in the dialog to the 'submit' button. At the bottom of the page, there is a note: 'Please install the software first, and when you open the installed software for the first time, please fill in the registration form shown to you and paste it into this input box.'

① Run **run_pGlycoGUI.bat**, fill in the forms, and then click **Copy to clipboard** button in the pGlyco3 License Dialog.

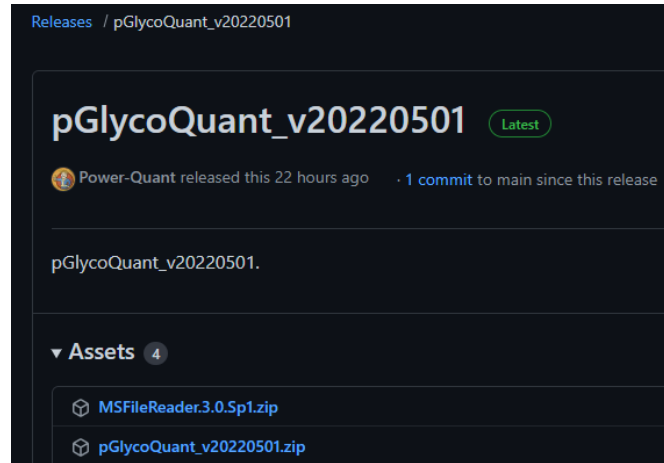
② Login <http://i.pfind.org/license/pGlyco3>, paste the information and **submit**.

③ Import the replied license (**pGlyco3.license**) file to the pGlyco3 License Dialog.

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant download

①



① Login <https://github.com/Power-Quant/pGlycoQuant/releases> and download the latest version at the top of the page.

If MSFileReader 3.0 has not been installed, please download **MSFileReader.3.0.Sp1.zip** and install it.

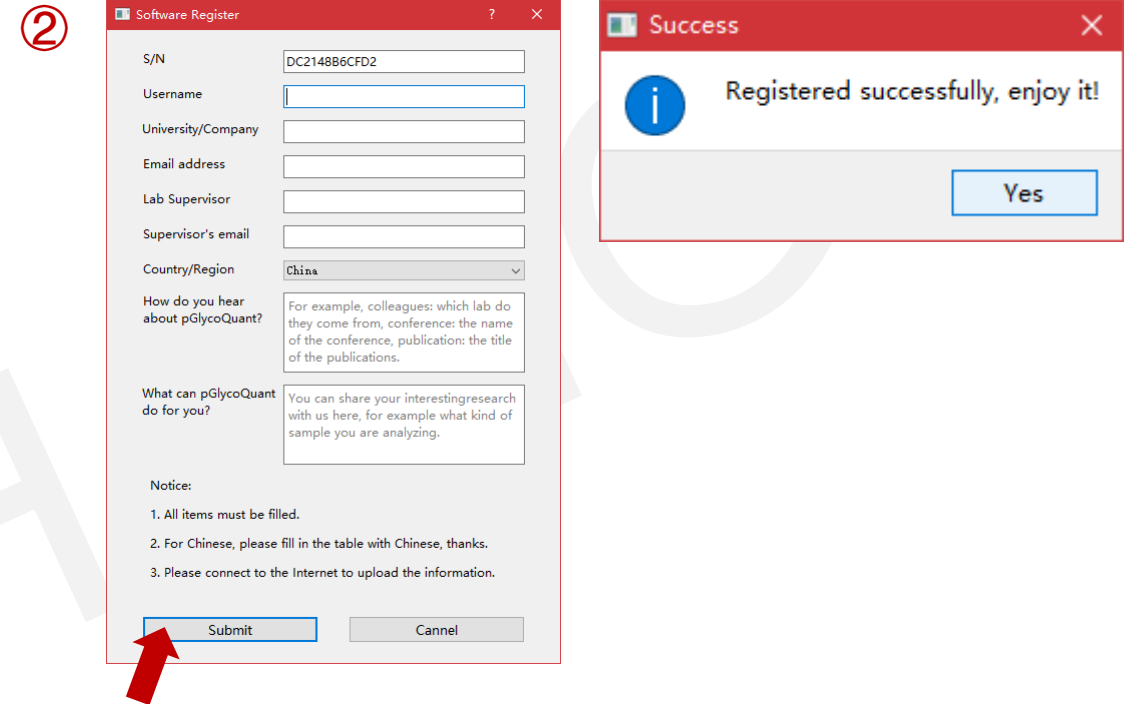
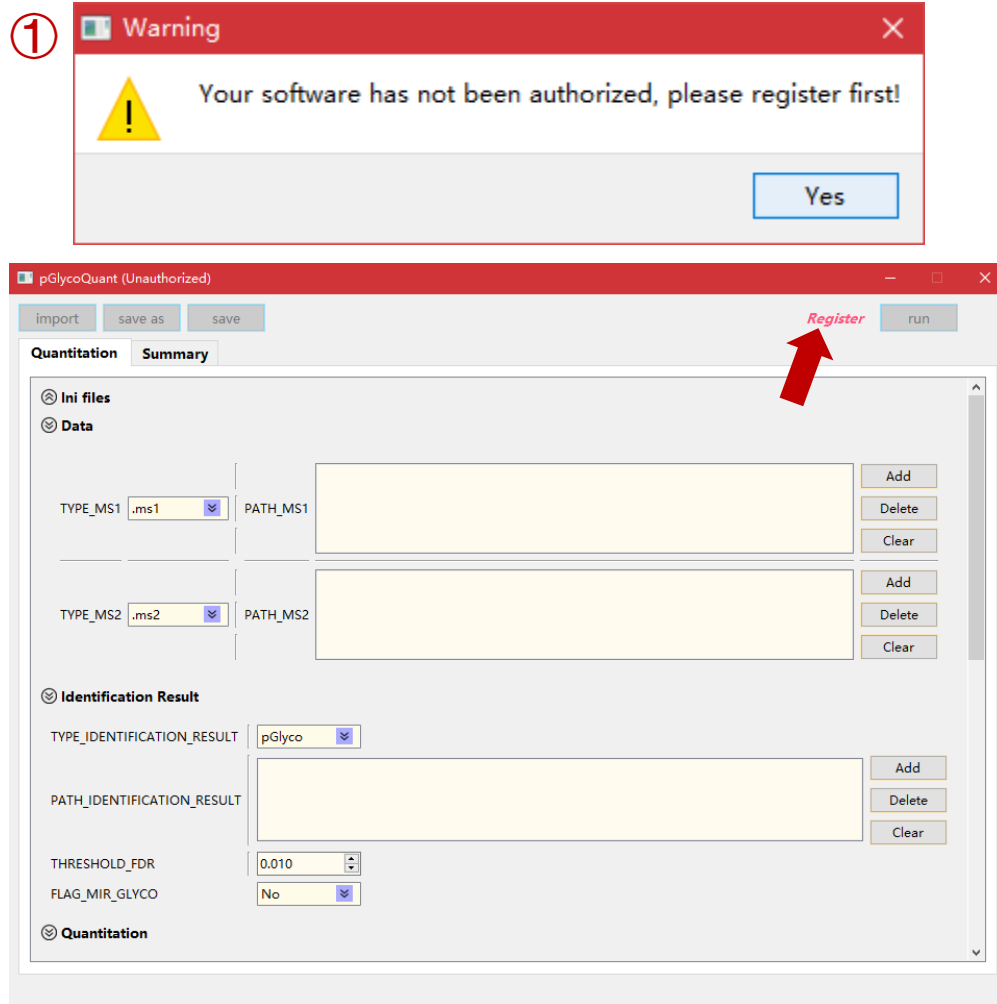
②



② The unzipped pGlycoQuant files.

2 Quantitation with pGlycoQuant

2.2 pGlycoQuant register



① Double click **pGlycoQuantUI.exe**, if software has not been authorized, click **Register** button, before that, make sure that your PC is linked to the Internet.

② Fill the register information and click **Submit**, then pGlycoQuant will be authorized.

pGlyco & pGlycoQuant

Manual for DDA Label Free Data



1 Identification with pGlyco

Label Free

1.1 pGlyco identification

Raw Protein Glycan Search

① Raw files
File Type: raw

② Fragmentation: HCD

③

File Name	Size
E:\Code\pQuant\article\ALL_data\LabelFree\1902-Hela-Labelfree-01.raw	1.78 GB
E:\Code\pQuant\article\ALL_data\LabelFree\1902-Hela-Labelfree-02.raw	1.73 GB

Add

Remove

Clear All

Load Parameters

- ① Load raw files.
- ② Set the **Fragmentation** as **HCD**.
- ③ Add the **Label Free** file.

1 Identification with pGlyco

Label Free

1.1 pGlyco identification

The screenshot shows the pGlyco3 software interface with the following settings and annotations:

- Annotation 1:** Points to the **Fasta:** field containing the path `E:\Code\pQuant\article\ALL_data\LabelFree\Human-H.sapiens-SP-1808_N2J_STC.fasta`.
- Annotation 2:** Points to the **Enzyme** section, where **Name:** is set to `Trypsin`, **Digestion:** is set to `specific`, **Digest N-Term:** is empty, **Digest C-Term:** is set to `KR`, and **Max Miss Cleavage:** is set to `2`.
- Annotation 3:** Points to the **Fixed Protein Modifications** list, which contains `Carbamidomethyl[C]`.
- Annotation 4:** Points to the **Max Var Mod on Peptide:** field, which is set to `3`.
- Other visible settings:**
 - Variable Protein Modifications:** `Oxidation[M]` and `Acetyl[ProteinN-term]` are listed.
 - Modification List:** A scrollable list of various modifications is shown on the right.
 - Peptide Length:** from `6` to `40`.
 - Peptide Mass:** from `600` to `4000`.

- ① Set the fasta database (The file could be downloaded from <https://github.com/expellir-arma/pGlycoQuant/>).
- ② Set the trypsin enzyme.
- ③ Set the fixed modification as Carbamidomethyl on Cys site. Set the variable modification as Acetyl on Protein N-Term and Oxidation on Met site.
- ④ Set the filter information.

1. Identification with pGlyco

Label Free

The screenshot shows the pGlyco3 software window with the following configuration:

- Glycan DB:** pGlyco-N-Human.gdb (indicated by ①)
- Glycan Type:** N-Glycan (indicated by ②)
- Fixed Glycan Modifications:** An empty list box.
- Variable Glycan Modifications:** H~pH (indicated by ③)
- Max Var Mod on Glycan:** 1
- Max Number of (Modified) Glycans to Search:** 100000

Navigation buttons (<<, >>) are present for both modification sections. A 'Convert GlycoWorkbench' button is also visible.

① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.

② Set the **Glycan Type** as **Glycan**.

③ Set the Glycan modification information.

1 Identification with pGlyco

Label Free

1.1 pGlyco identification

The screenshot shows the pGlyco3 software interface. It has a red title bar and a menu bar with 'Raw', 'Protein', 'Glycan', and 'Search'. Below the menu bar, there are several settings sections. On the left, under 'Precursor Tolerance', there is a text box with '± 4' and a dropdown menu set to 'ppm'. Below this, 'Number of Processes' is set to '5' in a dropdown. Further down, 'pGlycoSite: Localized Glycans Must Be in GDB' is checked. On the right, under 'Fragment Tolerance', there is a text box with '± 20' and a dropdown menu set to 'ppm'. Below this, 'Glycopeptide FDR' is set to '0.01' in a text box. In the center, there is a 'pGlycoNovo' section with a 'Run pGlycoNovo' checkbox. Below it, there are several 'Glyco:' labels followed by dropdown menus and 'Max:' text boxes. The first four are set to 'H', 'N', 'F', and 'A' with maximums of 20, 7, 5, and 4 respectively. The last three are empty. At the bottom of this section, 'Allow Max Glyco Gap' is set to '3' in a dropdown. At the very bottom, there is an 'Output Folder:' text box with the path 'E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco' and a 'Browse' button. To the right of the 'Output Folder' section are three buttons: 'Run', 'Save', and 'Stop'. Numbered annotations 1 through 6 are placed around the interface: 1 points to the Precursor Tolerance, 2 to the Fragment Tolerance, 3 to the Number of Processes, 4 to the Glycopeptide FDR, 5 to the Output Folder, and 6 to the Run button.

① Precursor Tolerance: ± 4 ppm

② Fragment Tolerance: ± 20 ppm

③ Number of Processes: 5

④ Glycopeptide FDR: 0.01

⑤ Output Folder: E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco

⑥ Run Save Stop

- ① Set the **Precursor Tolerance** as ± 4 ppm.
- ② Set the **Fragment Tolerance** as ± 20 ppm.
- ③ Set the **Number of Processes** according to your PC.
- ④ Set the **Glycopeptide FDR** as **0.01**.
- ⑤ Set the **Output Folder** for saving the identification results.
- ⑥ Click **Save** and **Run** buttons, the progress information will be shown in the command-line interface.

The screenshot shows a Windows command prompt window with the following text:

```
C:\WINDOWS\system32\cmd.exe
E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco> pGlyco3
E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco> pGlyco3
pGlyco3 Starting task ...
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\pGlyco3.exe "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process\pGlyco.cfg"
Process ID=14252: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\pGlyco3.exe "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process\pGlyco.cfg"
Process ID=14252: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\pGlyco3.exe "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process\pGlyco.cfg"
Process ID=14252: pGlyco
pGlyco2.2 (x64) from pFind Studio
Email: pfind@ict.ac.cn
Website: http://pfind.ict.ac.cn
*****
The 11 cases will expire in 2100-1-1
Process ID=14252: pParse <INFO>: pParse writes logs in E:\Code\pQuant\article\ALL_data\LabelFree\pParse\pGlycoLog.txt
Process ID=14252: pParse <INFO>: BEGIN PARAMETERS
Process ID=14252: pParse <INFO>: 01: check_active_incenter = 1
Process ID=14252: pParse <INFO>: 02: co-elute = 1
Process ID=14252: pParse <INFO>: 03: cut_similar_mono = 1
Process ID=14252: pParse <INFO>: 04: dataset = 1
Process ID=14252: pParse <INFO>: 05: datapath1 = E:\Code\pQuant\article\ALL_data\LabelFree\1902-Hela-LabelFree-02.ms1
Process ID=14252: pParse <INFO>: 06: delete_mon = 0
Process ID=14252: pParse <INFO>: 07: dia_mode = 0
Process ID=14252: pParse <INFO>: 08: dia_mode_to_filter_by_selectedMS2ScanNoGet = 1
Process ID=14252: pParse <INFO>: 09: input_format = ms1
Process ID=14252: pParse <INFO>: 10: intensity = 1
Process ID=14252: pParse <INFO>: 11: ipr_file = IPR.txt
Process ID=14252: pParse <INFO>: 12: isolation_width = 2.000000
Process ID=14252: pParse <INFO>: 13: logfilepath = E:\Code\pQuant\article\ALL_data\LabelFree
Process ID=14252: pParse <INFO>: 14: m/z = 5
Process ID=14252: pParse <INFO>: 15: mazy_model = 4
Process ID=14252: pParse <INFO>: 16: mazy_threshold = -0.500000
Process ID=14252: pParse <INFO>: 17: mazy = 20.000000
Process ID=14252: pParse <INFO>: 18: mazy_lpm = 1
Process ID=14252: pParse <INFO>: 19: output_mz = 1
Process ID=14252: pParse <INFO>: 20: output_sif = 1
Process ID=14252: pParse <INFO>: 21: outputpath =
Process ID=14252: pParse <INFO>: 22: recalibrate_window = 7.000000
```

1 Identification with pGlyco

Label Free

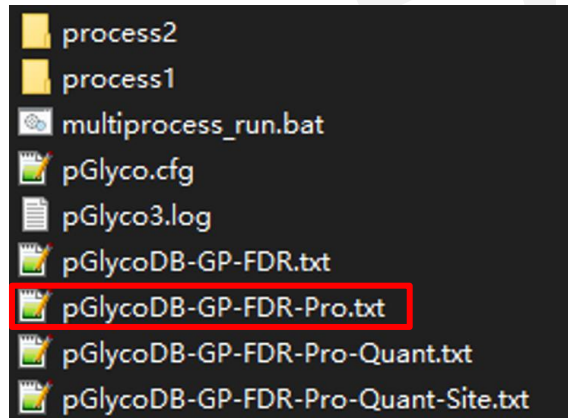
1.2 identification results

①

```
Process ID=14252: [XIC] Loading pGlyco results ...
Process ID=14252: [XIC] RT window is [-120.0, +120.0] seconds
Process ID=14252: [XIC] Indexing e:\code\pquant\article\all_data\labelfree\1902-hela-labelfree-02.pf1
Process ID=14252: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\pGlycoSite.exe "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process2\pGlyco.cfg"
Process ID=14252: Already registered!=====
Process ID=14252: [pGlycoSite] Glycosylation site localization finished!=====
Process ID=25040:
Process ID=25040: [pGlyco] E:\Code\pQuant\article\ALL_data\LabelFree\1902-Hela-Labelfree-01_HCDFT.mgf finished!
Process ID=25040: Timing: 1040.64 seconds=====
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\gpPercolator.exe -p "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlyco.cfg"
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\pGlycoFDR.exe -p "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlyco.cfg"
Process ID=25040: E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlycoDB-GP-Raw1-FDR.txt
Process ID=25040: 2569 GPSMs at 1.0% FDR
Process ID=25040: 12529 GPSMs at 100.0% FDR
Process ID=25040: merge into E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlycoDB-GP-FDR.txt
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\pGlycoProinfer.exe -p "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlyco.cfg"
Process ID=25040: Reading pGlyco results ...
Process ID=25040: Inferring proteins ...
Process ID=25040: End inference
Process ID=25040: E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlycoDB-GP-FDR-Pro.txt
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\XIC.exe -p "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlyco.cfg"
Process ID=25040: [XIC] Smoothing window = 21
Process ID=25040: [XIC] Smoothing method = savgol_filter
Process ID=25040: [XIC] Loading pGlyco results ...
Process ID=25040: [XIC] RT window is [-120.0, +120.0] seconds
Process ID=25040: [XIC] Indexing e:\code\pquant\article\all_data\labelfree\1902-hela-labelfree-01.pf1
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\pGlycoSite.exe "E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlyco.cfg"
Process ID=25040: Already registered!
Process ID=25040: [pGlycoSite] Glycosylation site localization finished!
[pGlyco] All results are merged!
[pGlyco] Running time = 39.8 minutes.
[pGlyco] Task completed!
```

- ① The completed information in the command-line interface.
- ② The identification result file used for quantitation.

②



2 Quantitation with pGlycoQuant

Label Free

2.1 pGlycoQuant quantitation

pGlycoQuant (Authorized)

import save as save run

Quantitation Summary

Ini files

PATH_AA E:/Code/pQuant/Core/pGlycoQuant20220512/ini/ini_pGlyco/aa.ini Browser

PATH_ELEMENT E:/Code/pQuant/Core/pGlycoQuant20220512/ini/ini_pGlyco/element.ini Browser

PATH_GLYCO E:/Code/pQuant/Core/pGlycoQuant20220512/ini/ini_pGlyco/glyco.ini Browser

PATH_MOD E:/Code/pQuant/Core/pGlycoQuant20220512/ini/ini_pGlyco/modification.ini Browser

PATH_LINKER E:/Code/pQuant/Core/pGlycoQuant20220512/ini/ini_pGlyco/xlink.ini Browser

PATH_GDB E:/Code/pQuant/P/pGlycoQuant_v20220501/ini/N-common_structure.gdb Browser

Data

TYPE_MS1 .raw PATH_MS1 E:/Code/pQuant/article/ALL_data/001_LabelFree/1902-Hela-Labelfree-01.raw
E:/Code/pQuant/article/ALL_data/001_LabelFree/1902-Hela-Labelfree-02.raw Add Delete Clear

TYPE_MS2 .ms2 PATH_MS2 Add Delete Clear

① Ensure that the ini file paths are valid.

② Set the **TYPE_MS1** as **.raw** and fill the raw files into the **PATH_MS1** blank.

2 Quantitation with pGlycoQuant

Label Free

2.1 pGlycoQuant quantitation

pGlycoQuant (Authorized)

import save as save run

Quantitation Summary

Identification Result

① TYPE_IDENTIFICATION_RESULT pGlyco

② PATH_IDENTIFICATION_RESULT E:/Code/pQuant/article/ALL_data/001_LabelFree/pGlyco/pGlycoDB-GP-FDR-Pro.txt Add Delete Clear

THRESHOLD_FDR 0.010

③ FLAG_MIR_GLYCO No

Quantitation

④ TYPE_QUANT DDA LabelFree

RI_PPM_HALF_WIN_ACCURACY_PEAK 1000.00

RI_MASS_REPORT_ION 127.11, 130.11

DDALL_RT_HALF_WIN_IN_MIN 2.00

DDALL_PPM_HALF_WIN_ACCURACY_PEAK 20.00

DDALL_LABEL_INFO 2[NONE]AA:R:N:15N&AA:R:C:13C&AA:K:C:13C&AA:K:N:15N

DDALL_FLAG_CALIBRATION_180 No

- ① Set **TYPE_IDENTIFICATION_RESULT** as **pGlyco**
(For other identification software results like Byonic and MSFragger, Byonic and MSFragger glyco-N options also can be chosen).
- ② Put the identification result file **pGlycoDB-GP-FDR-Pro.txt** here and set **FDR** as **0.01**.
- ③ If **MIR** quantitation is needed, the **FLAG_MIR_GLYCO** can be set as **Yes**.
- ④ Set the **TYPE_QUANT** as **DDA LabelFree**.

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

Label Free

pGlycoQuant (Authorized)

import save as save run

Quantitation Summary

RI_PPM_HALF_WIN_ACCURACY_PEAK 1000.00

RI_MASS_REPORT_ION 127.11, 130.11

DDALL_RT_HALF_WIN_IN_MIN 2.00

DDALL_PPM_HALF_WIN_ACCURACY_PEAK 20.00

DDALL_LABEL_INFO 2[NONE]AA:R:N:15N&AA:R:C:13C&AA:K:C:13C&AA:K:N:15N

DDALL_FLAG_CALIBRATION_18O No

① DDALF_RT_HALF_WIN_IN_MIN 2.00

② DDALF_PPM_HALF_WIN_ACCURACY_PEAK 20.00

③ DDALF_FLAG_DECOY No

④ DDALF_MIR_FLAG_RT_CALIBRATION No

DDALF_MIR_RT_SHIFT_A 500.00

DDALF_MIR_RT_HALF_WIN_IN_MIN 2.00

Export ⑤

PATH_EXPORT E:/Code/pQuant/article/ALL_data/001_LabelFree/pQuant2 Browser

FLAG_CREATE_NEW_FOLDER Yes

① Set **DDALF_RT_WIN_IN_MIN** as 2.00 minutes (default setting).

② Set **DDALF_PPM_HALF_WIN_ACCURACY_PEAK** as 20.00 ppm (default setting).

③ The **DDALF_FLAG_DECOY** can be set as **Yes** to run the **FQR** for Label Free raws.

④ If **FLAG_MIR_GLYCO** is set as **Yes**:
DDALF_MIR_FLAG_RT_CALIBRATION means the switch for manually adjusting RT_{SA} , if “**Yes**” was chosen, **DDALF_MIR_RT_SHIFT_A** (second) should be set.

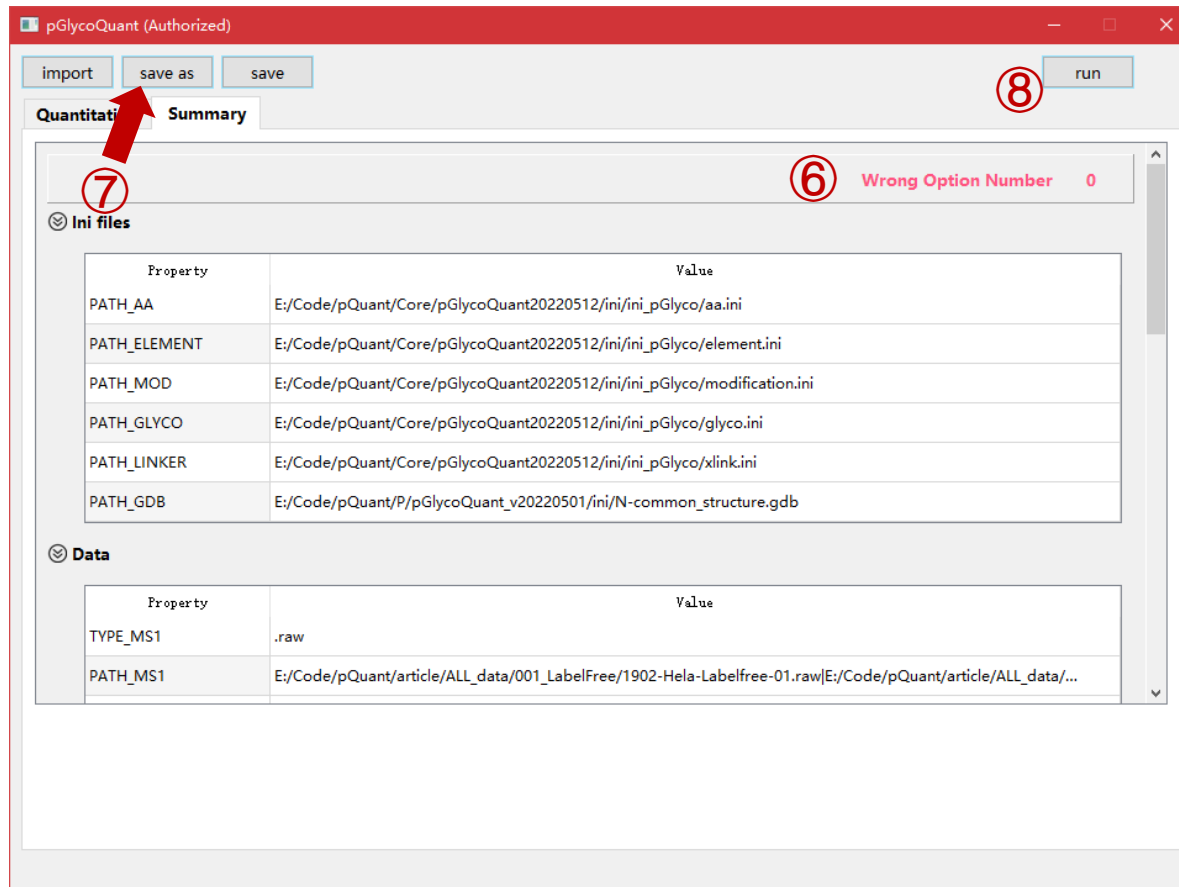
Set **DDALF_MIR_RT_HALF_WIN_IN_MIN** as 2.00 minutes (default setting).

⑤ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

Label Free

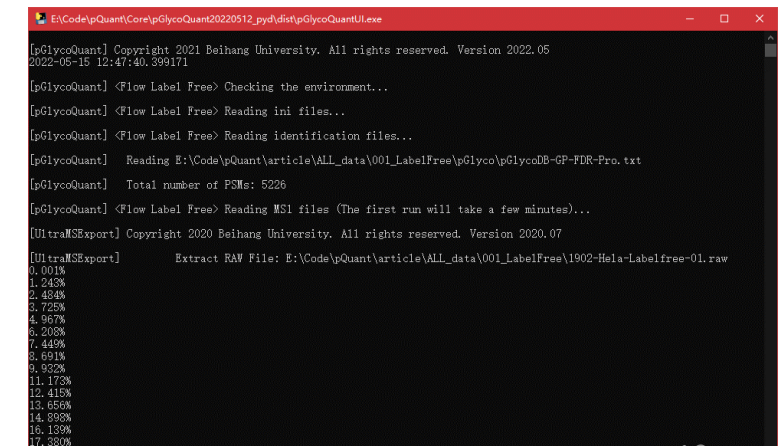
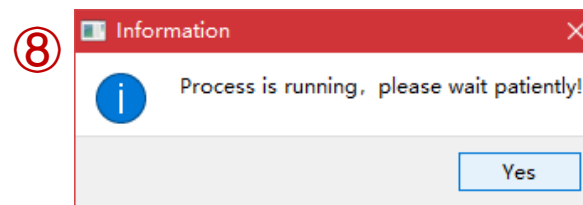
2.1 pGlycoQuant quantitation



⑥ Click **Summary** button and make sure that the **Wrong Option Number** is 0.

⑦ Then click **save as** button to save the config file as a config file.

⑧ Click **run** button to start the quantitation, the progress information will be shown in the command-line interface.



2 Quantitation with pGlycoQuant

Label Free

2.2 quantitation results

①

```
E:\Code\pQuant\Core\pGlycoQuant20220512_py\d\dist\pGlycoQuantUI.exe
45.962%
53.617%
61.271%
68.925%
76.579%
84.233%
91.887%
99.541%
100.0%

[pGlycoQuant] <Function Quantitation> Getting decoy evidences for references...
0.038%
7.692%
15.346%
23.000%
30.654%
38.308%
45.962%
53.617%
61.271%
68.925%
76.579%
84.233%
91.887%
99.541%
100.0%

[pGlycoQuant] <Function Quantitation> Getting decoy evidences for samples...
0.038%
7.692%
15.346%
23.000%
30.654%
38.308%
45.962%
53.617%
61.271%
68.925%
76.579%
84.233%
91.887%
99.541%
100.0%

[pGlycoQuant] <Function FQR> Calculate FQR thresholds and filter quantitative results...

[pGlycoQuant] <Flow Label Free> Infering...

[pGlycoQuant] Finished!
```

②

- cfg_labelfree.txt
- pGlycoQuant.modification.list
- pGlycoQuant.protein.list
- pGlycoQuant.site.list
- pGlycoQuant.spectra.info
- pGlycoQuant.spectra.list

① The completed information.

② The quantitation results. Please open the files with Excel.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
GlySpec	PepSpec	RawName	Scan	RT	PrecursorMz	PrecursorChg	Rank	Peptide	Mod	PeptideMz	GlycanH	GlycanCon	PlausibleSt	GlyD	GlyFrag	GlyMass	GlySite	TotalScore	PepScore	
1	1902-Hela 1902-Hela 1902-Hela	4911	1317.914	2396.956	1195.982	2	1	AAAGSLR		688.7377	8.2.0.0.0	H8(N)2	(N)N(H)H	14676.0	1.0.0.0.0	1702.581	3	55.81661	28.12736	
2	1902-Hela 1902-Hela 1902-Hela	15356	4121.43	3447.341	1149.785	3	1	TLSPSPSET9.Carbam		1623.764	6.3.0.0.1	H6(N)2	(N)N(H)H	1823.574	1.0.0.0.0	1823.574	10	35.33359	27.7551	
4	1902-Hela 1902-Hela 1902-Hela	33563	9020.257	4087.664	1022.871	4	1	ATYGHYAPGEEHDI		2643.127	3.4.0.1.0	H3(N)4F1	(N)FYNH	3180.0	1.0.0.0.0	1444.534	1	13.39333	13.34168	
5	1902-Hela 1902-Hela 1902-Hela	29913	8039.566	2716.157	1358.582	2	1	DSLSTATNKK		1175.627	7.2.0.0.0	H7(N)2	(N)N(H)H	11421.0	1.0.0.0.0	1540.529	6	15.34538	81.6648	
6	1902-Hela 1902-Hela 1902-Hela	47534	12770.07	3440.505	1147.507	3	1	ICTSISGDL2.Carbam		1899.975	7.2.0.0.0	H7(N)2	(N)N(H)H	11421.0	1.0.0.0.0	1540.529	1	82.41943	88.36935	
7	1902-Hela 1902-Hela 1902-Hela	43617	11715.8	3591.435	1197.817	3	1	VHYGSLMDGTFDSS		2212.96	6.2.0.0.0	H6(N)2	(N)N(H)H	11463.0	1.0.0.0.0	1378.476	4	12.46524	12.25986	
8	1902-Hela 1902-Hela 1902-Hela	35087	9423.893	3258.381	1086.799	3	1	SASTLAQALHWT		1555.796	8.2.0.0.0	H8(N)2	(N)N(H)H	10352.0	1.0.0.0.0	1702.581	3	101.1777	99.31352	
9	1902-Hela 1902-Hela 1902-Hela	23187	6233.761	2691.202	1346.105	2	1	KYRLPK		989.619	8.2.0.0.0	H8(N)2	(N)N(H)H	14636.0	1.0.0.0.0	1702.581	7	41.41771	30.39843	
10	1902-Hela 1902-Hela 1902-Hela	34850	9365.915	3011.23	1506.119	2	1	VIETWAWK		1146.594	9.2.0.0.0	H9(N)2	(N)N(H)H	10335.0	1.0.0.0.0	1864.634	3	30.92117	19.31954	
11	1902-Hela 1902-Hela 1902-Hela	26333	7079.023	3634.581	1212.198	3	1	VASVININI18.Carbam		2027.993	4.4.0.1.0	H4(N)4F1	(N)FYNH	3155.0	1.0.0.0.0	1606.587	10	15.81626	11.06153	
12	1902-Hela 1902-Hela 1902-Hela	22591	6072.635	2628.13	1314.569	2	1	LININPK		925.5465	8.2.0.0.0	H8(N)2	(N)N(H)H	10352.0	1.0.0.0.0	1702.581	7	35.83687	20.86584	
13	1902-Hela 1902-Hela 1902-Hela	39030	10487.29	2737.074	1369.041	2	1	QWNTSGFK		1196.548	7.2.0.0.0	H7(N)2	(N)N(H)H	11421.0	1.0.0.0.0	1540.529	3	17.84072	10.33826	
14	1902-Hela 1902-Hela 1902-Hela	26231	7051.652	3306.429	1102.815	3	1	JISGVYLADHSGAFHW		1765.898	7.2.0.0.0	H7(N)2	(N)N(H)H	11421.0	1.0.0.0.0	1540.529	1	53.67543	54.75203	
15	1902-Hela 1902-Hela 1902-Hela	5681	1523.015	3472.291	1158.102	3	1	JACSTJTS3.Carbam		1607.654	9.2.0.0.0	H9(N)2	(N)N(H)H	10335.0	1.0.0.0.0	1864.634	1	28.84277	9.65945	
16	1902-Hela 1902-Hela 1902-Hela	6744	1807.54	2213.903	1107.455	2	1	SLISSTAR		835.4268	6.2.0.0.0	H6(N)2	(N)N(H)H	11510.0	1.0.0.0.0	1378.476	4	57.97362	46.13034	
17	1902-Hela 1902-Hela 1902-Hela	61666	16558.01	3410.352	1137.456	3	1	QQVWPGWVWQPIR		1748.829	5.0.0.1.0	H5(N)3	(N)N(H)H	10451.0	1.0.0.0.0	1661.521	12	23.875	17.67967	
18	1902-Hela 1902-Hela 1902-Hela	21632	5818.444	3770.521	1257.512	3	1	GYVQSEAGSHLQR		1710.783	5.4.1.1.0	H5(N)4	(N)FYNH	2373.0	1.0.0.0.0	2059.735	4	35.89245	19.07819	
19	1902-Hela 1902-Hela 1902-Hela	37105	9972.603	3050.284	1525.646	2	1	ALGFEIATQALGR		1347.702	8.2.0.0.0	H8(N)2	(N)N(H)H	10345.0	1.0.0.0.0	1702.581	6	28.47994	17.16471	
20	1902-Hela 1902-Hela 1902-Hela	22540	6058.86	2808.154	936.723	3	1	EIATDILTK		1105.574	8.2.0.0.0	H8(N)2	(N)N(H)H	10258.0	1.0.0.0.0	1702.581	2	15.52441	9.66117	
21	1902-Hela 1902-Hela 1902-Hela	27278	7331.399	2996.235	1448.621	2	1	VGGQALTVK		1193.652	8.2.0.0.0	H8(N)2	(N)N(H)H	10920.0	1.0.0.0.0	1702.581	6	82.7849	65.68707	
22	1902-Hela 1902-Hela 1902-Hela	3003	803.8361	2491.947	1246.477	2	1	FJHTR		789.3638	8.2.0.0.0	H8(N)2	(N)N(H)H	10352.0	1.0.0.0.0	1702.581	2	39.82172	18.64579	
23	1902-Hela 1902-Hela 1902-Hela	30176	8110.741	3224.278	1075.431	3	1	EQUITLDR		1238.649	7.3.0.0.1	H7(N)3	(N)N(H)H	13430.0	1.0.0.0.0	1985.627	4	32.60493	17.27136	
24	1902-Hela 1902-Hela 1902-Hela	22522	6054.02	2628.13	1314.569	2	1	LININPK		925.5465	8.2.0.0.0	H8(N)2	(N)N(H)H	10352.0	1.0.0.0.0	1702.581	7	36.49143	24.66089	
25	1902-Hela 1902-Hela 1902-Hela	37905	10185.56	3961.71	1321.241	3	1	TLTVDVNTWVNSHIAL		2097.072	9.2.0.0.0	H9(N)2	(N)N(H)H	14542.0	1.0.0.0.0	1964.634	6	17.41539	9.41301	
26	1902-Hela 1902-Hela 1902-Hela	41167	11058.03	2611.214	871.0763	3	1	TISTFQALVEHK		1572.838	3.2.0.1.0	H3(N)2	(N)FYNH	3355.0	1.0.0.0.0	1038.375	2	31.04382	29.92218	
27	1902-Hela 1902-Hela 1902-Hela	38707	10400.71	4033.613	1345.209	3	1	VHYGTLDTGTFDST		2169.977	9.2.0.0.0	H9(N)2	(N)N(H)H	14673.0	1.0.0.0.0	1864.634	4	39.80104	31.76965	
28	1902-Hela 1902-Hela 1902-Hela	10247	2748.709	2998.048	866.6875	3	1	ENQHSYSLK		1219.57	6.2.0.0.0	H6(N)2	(N)N(H)H	11510.0	1.0.0.0.0	1378.476	4	51.20717	39.91228	
29	1902-Hela 1902-Hela 1902-Hela	47842	12853.49	4106.816	1027.459	4	1	TQDQVQREFAEQLD		2728.332	6.2.0.0.0	H6(N)2	(N)N(H)H	11510.0	1.0.0.0.0	1378.476	19	57.50054	70.20237	
30	1902-Hela 1902-Hela 1902-Hela	7882	2113.681	2478.088	826.7008	3	1	LIDNTEK		1074.579	4.3.0.1.0	H4(N)3	(N)FYNH	3304.0	1.0.0.0.0	1403.507	5	27.68722	33.02479	
31	1902-Hela 1902-Hela 1902-Hela	16731	4492.785	2593.035	1297.021	2	1	FSSSSSSLEEK		1214.554	6.2.0.0.0	H6(N)2	(N)N(H)H	14693.0	1.0.0.0.0	1378.476	2	53.50094	60.08892	
32	1902-Hela 1902-Hela 1902-Hela	52885	14211.48	4233.873	1059.224	4	1	LPJSTIVPEVGYLFT		2531.293	8.2.0.0.0	H8(N)2	(N)N(H)H	10258.0	1.0.0.0.0	1702.581	3	25.87243	22.33618	

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2 Quantitation with pGlycoQuant

Label Free

2.2 quantitation results --- MIR results

	AL	AM	AN	AO	AP
1	Genes	ProSites	Supp_Info	Empty_Sep	Intensity(2C1r
2753	IGHG2	176	null		1.93E+08
2754	IGHG2	176	null		6.07E+09
2755	IGHG1	180	null		4.94E+08
2756	IGHG2	176	null		1.05E+08
2757	IGHG1	180	H(5)N(4)_(N(N(H(H(H))(H(N)(N(H))))))		9.46E+08
2758	IGHG1	180	H(5)N(4)A(1)_(N(N(H(H(H))(H(N)(N(H(A))))))		1417575
2759	IGHG1	180	H(4)N(4)_(N(N(H(H(N))(H(N(H))))))		5083684
2760	IGHG1	180	H(4)N(4)A(1)_(N(N(H(H(N))(H(N(H(A))))))		3659559
2761	IGHG1	180	H(5)N(4)_(N(N(H(H(H))(H(N(H))))))		28256694
2762	IGHG1	180	H(5)N(4)A(1)_(N(N(H(H(H))(H(N(H(A))))))		1417575
2763	IGHG1	180	H(5)N(4)A(2)_(N(N(H(H(N(H(A))))(H(N(H(A))))))		6163931
2764	IGHG1	180	H(5)N(4)_(N(N(H(H(N(H))))(H(N(H))))))		4.91E+08
2765	IGHG1	180	H(5)N(4)A(1)_(N(N(H(H(N(H))))(H(N(H(A))))))		68903228

The “Supp_Info” column shows the information of MIR quantitation: “null” means the quantitation of identified result from pGLyco, and “ H(5)N(4)A(1)_(N(N(H(H(H))(H(N)(N(H(A)))))) ” means the quantitated candidate glycopeptide from the subnet.

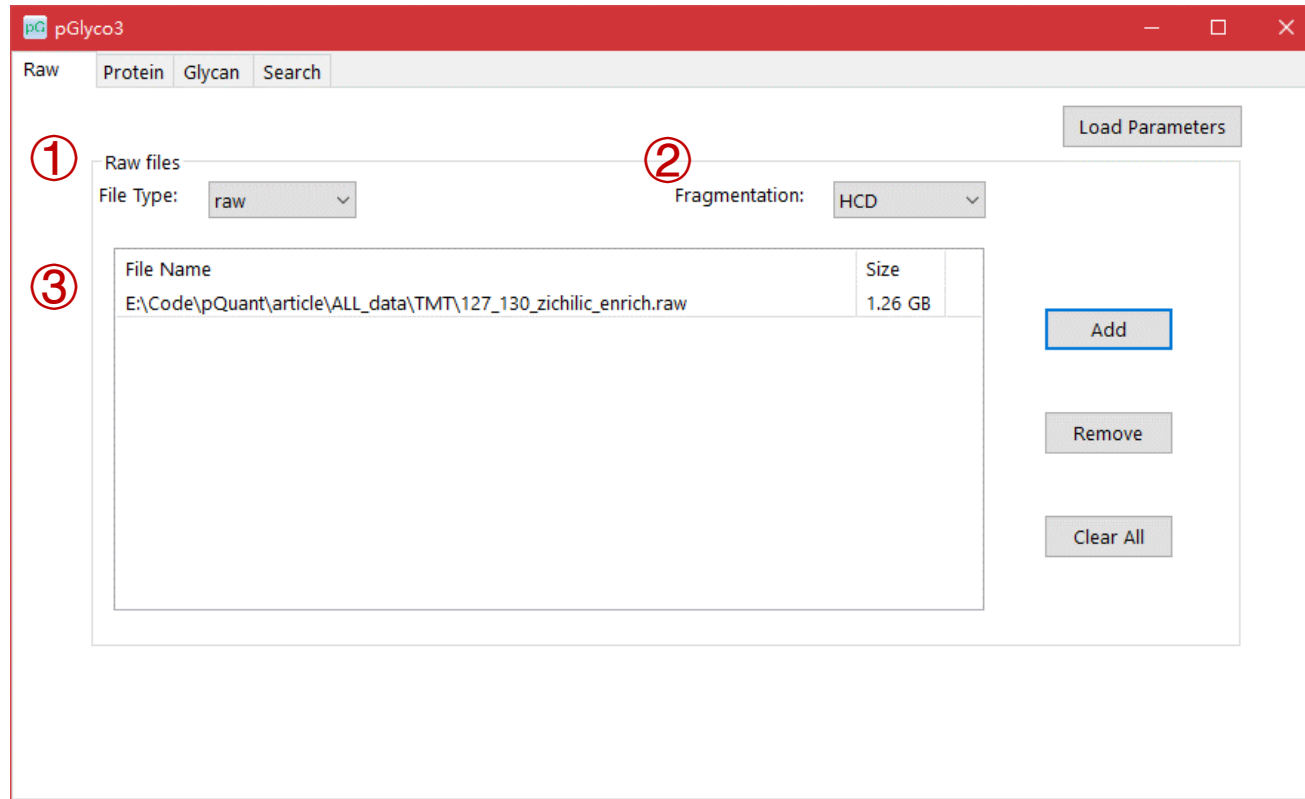


pGlyco & pGlycoQuant

Manual for TMT Data



1. Identification with pGlyco



- ① Load raw files.
- ② Set the **Fragmentation** as **HCD**.
- ③ Add the **TMT** raw file.

1. Identification with pGlyco

The screenshot shows the pGlyco3 software interface with the following settings and annotations:

- Annotation 1:** Points to the **Fasta:** text field containing the path `E:\Code\pQuant\article\ALL_data\TMT\Human-H.sapiens-SP-1808_N2J_STC.fasta`.
- Annotation 2:** Points to the **Enzyme** section, where the **Name:** is set to `Trypsin`.
- Annotation 3:** A red box highlights the **Fixed Protein Modifications** and **Variable Protein Modifications** sections. The fixed modifications listed are `Carbamidomethyl[C]`, `TMT6plex[AnyN-term]`, and `TMT6plex[K]`. The variable modifications listed are `Oxidation[M]` and `Acetyl[ProteinN-term]`.
- Annotation 4:** Points to the **Max Var Mod on Peptide:** field, which is set to `3`.

Other visible settings include:

- Digest N-Term:** (empty)
- Digest C-Term:** `KR`
- Max Miss Cleavage:** `2`
- Digestion:** `specific`
- Modification List:** A scrollable list of modifications including `TMT2plex[K]`, `TMT2plex[S]`, `TMT2plex[T]`, `TMT2plex[ProteinN-term]`, `TMT6plex[AnyN-term]`, `TMT6plex[H]`, `TMT6plex[K]`, `TMT6plex[S]`, `TMT6plex[T]`, `TMT6plex[ProteinN-term]`, `TNBS[AnyN-term]`, `TNBS[K]`, `Thiadiazole[C]`, `Thiazolidine[AnyN-termC]`, `Thioacyl[AnyN-term]`, `Thioacyl[K]`, `Thiophos-S-S-biotin[S]`, `Thiophos-S-S-biotin[T]`, and `Thiophos-S-S-biotin[M]`.
- Filter:** (empty)
- Update** button
- Peptide Length:** from `6` to `40`
- Peptide Mass:** from `600` to `4000`

① Set the fasta database (The file could be downloaded from <https://github.com/expellir-arma/pGlycoQuant/>).

② Set the trypsin enzyme.

③ Set the modification information like the left panel.

④ Set the filter information.

1. Identification with pGlyco

The screenshot shows the pGlyco3 application window with a red title bar. It has four tabs: 'Raw', 'Protein', 'Glycan', and 'Search'. The 'Glycan' tab is active. At the top, there are two dropdown menus: 'Glycan DB:' set to 'pGlyco-N-Human.gdb' (marked with a red circled 1) and 'Glycan Type:' set to 'N-Glycan' (marked with a red circled 2). To the right of these is a 'Convert GlycoWorkbench' button. Below these are two sections for glycan modifications. The 'Fixed Glycan Modifications' section has an empty list box on the left and controls on the right: '<<' and '>>' buttons, a 'Glyco:' dropdown set to 'N', and a 'Modified as:' dropdown. The 'Variable Glycan Modifications' section (marked with a red circled 3) has a list box containing 'H~pH' on the left and controls on the right: '<<' and '>>' buttons, a 'Glyco:' dropdown set to 'H', and a 'Modified as:' dropdown set to 'pH'. At the bottom, there are two input fields: 'Max Var Mod on Glycan:' with the value '1' and 'Max Number of (Modified) Glycans to Search:' with the value '100000'.

① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.

② Set the **Glycan Type** as **Glycan**.

③ Set the Glycan modification information.

1. Identification with pGlyco

The image shows the pGlyco3 software interface with several numbered annotations:

- ①** Precursor Tolerance: ± 4 ppm
- ②** Fragment Tolerance: ± 20 ppm
- ③** Number of Processes: 5
- ④** Glycopeptide FDR: 0.01
- ⑤** Output Folder: E:\Code\pQuant\article\ALL_data\TMT\pGlyco
- ⑥** Run button

Other visible settings include:

- ☐ Percolator
- ☐ FMM for Peptide FDR
- ☒ pGlycoSite: Localized Glycans Must Be in GDB
- pGlycoNovo** section:
 - ☐ Run pGlycoNovo
 - Glyco: H Max: 20
 - Glyco: N Max: 7
 - Glyco: F Max: 5
 - Glyco: A Max: 4
 - Glyco: (empty) Max: (empty)
 - Glyco: (empty) Max: (empty)
 - Glyco: (empty) Max: (empty)
 - Glyco: (empty) Max: (empty)
- Allow Max Glyco Gap: 3

- ① Set the **Precursor Tolerance** as ± 4 ppm.
- ② Set the **Fragment Tolerance** as ± 20 ppm.
- ③ Set the **Number of Processes** according to your PC.
- ④ Set the **Glycopeptide FDR** as **0.01**.
- ⑤ Set the **Output Folder** for saving the identification results.
- ⑥ Click **Save** and **Run** buttons, the progress information will be shown in the command-line interface.

```
E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615>cd bin
E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlyco3GUI.exe
Already registered!
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.cfg
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.cfg
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.cfg
[pGlyco] Starting task ...
Process ID=14616: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\rem *****
*****
Process ID=14616: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\parse_raw.exe "E:\Code\pQuant\article\ALL_data\SI
LAC\process\pGlyco3.cfg"
Process (pid=30324) has been killed Row> 2.50%
Process (pid=14616) has been killed
Process (pid=10172) has been killed
Process 1 has been killed
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.cfg
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.cfg
[pGlyco] Starting task ...
Process ID=14168: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\rem *****
*****
Process ID=14168: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\parse_raw.exe "E:\Code\pQuant\article\ALL_data\SI
LAC\process\pGlyco3.cfg"
Process ID=14168: [pGlyc *****
*****
Process ID=14168: { pParse2.2 (x64) from pFind Studio
Email : pfind@ict.ac.cn
Website: http://pfind.ict.ac.cn
*****
*****
Process ID=14168: The license will expire in 2100-1-1
Process ID=14168: [pParse] CINFO: - pParse writes logs in E:\Code\pQuant\article\ALL_data\pGlyco\pParsePlusLog.txt
Process ID=14168: [pParse] CINFO: - ----- BEGIN PARAMETERS -----
Process ID=14168: [pParse] CINFO: - 01: check_activationcenter = 1
```

1. Identification with pGlyco

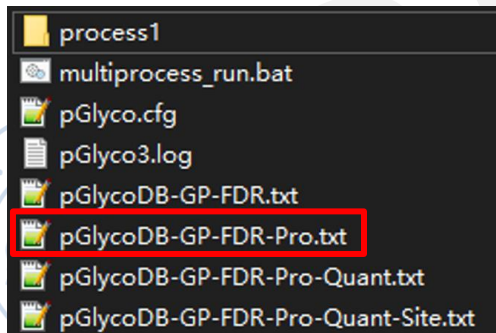
①

```
C:\WINDOWS\system32\cmd.exe
Process ID=03924: Timing: 353.391 seconds=====]
Process ID=03924: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>gpPercolator.exe -p "E:\Code\pQuant\
\article\ALL_data\TMT\pGlyco\process1\pGlyco.cfg"
Process ID=03924: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlycoFDR.exe -p "E:\Code\pQuant\ar
ticle\ALL_data\TMT\pGlyco\process1\pGlyco.cfg"
Process ID=03924: E:\Code\pQuant\article\ALL_data\TMT\pGlyco\process1\pGlycoDB-GP-raw1-FDR.txt
Process ID=03924: 119 GPSMs at 1.0% FDR
Process ID=03924: 7066 GPSMs at 100.0% FDR
Process ID=03924: merge into E:\Code\pQuant\article\ALL_data\TMT\pGlyco\process1\pGlycoDB-GP-FDR-Pro.txt
Process ID=03924: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlycoProInfer.exe -p "E:\Code\pQua
nt\article\ALL_data\TMT\pGlyco\process1\pGlyco.cfg"
Process ID=03924: Reading pGlyco results ...
Process ID=03924: Inferring proteins ...
Process ID=03924: End inference
Process ID=03924: E:\Code\pQuant\article\ALL_data\TMT\pGlyco\process1\pGlycoDB-GP-FDR-Pro.txt
Process ID=03924: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>XIC.exe -p "E:\Code\pQuant\article\
ALL_data\TMT\pGlyco\process1\pGlyco.cfg"
Process ID=03924: [XIC] Smoothing window = 21
Process ID=03924: [XIC] Smoothing method = savgol_filter
Process ID=03924: [XIC] Loading pGlyco results ...
Process ID=03924: [XIC] RT window is [-120.0, +120.0] seconds
Process ID=03924: [XIC] Indexing e:\code\pquant\article\all_data\tmt\127_130_zichilic_enrich.pfl
Process ID=03924: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlycoSite.exe "E:\Code\pQuant\arti
cle\ALL_data\TMT\pGlyco\process1\pGlyco.cfg"
Process ID=03924: Already registered!
Process ID=03924: [pGlycoSite] Glycosylation site localization finished!
[pGlyco] All results are merged!
[pGlyco] Running time = 17.5 minutes.
[pGlyco] Task completed!
```

① The completed information in the command-line interface.

② The identification results.

②



2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

①

②

① Ensure that the ini file paths are valid.

② Set the **TYPE_MS2** as **.raw** and fill the raw files into the **PATH_MS2** blank.

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

pGlycoQuant (Authorized)

import save as save run

Quantitation Summary

Identification Result

① TYPE_IDENTIFICATION_RESULT pGlyco

② PATH_IDENTIFICATION_RESULT E:/Code/pQuant/article/ALL_data/002_TMT/pGlyco/pGlycoDB-GP-FDR-Pro.txt Add Delete Clear

THRESHOLD_FDR 0.010

FLAG_MIR_GLYCO No

③ Quantitation

TYPE_QUANT ReportIon

RI_PPM_HALF_WIN_ACCURACY_PEAK 5.00

RI_MASS_REPORT_ION 126.127726,127.124761,128.134436,129.131471,130.141145,131.138180

DDALL_RT_HALF_WIN_IN_MIN 2.00

DDALL_PPM_HALF_WIN_ACCURACY_PEAK 20.00

DDALL_LABEL_INFO 2[NONE][AA:R:N:15N&AA:R:C:13C&AA:K:C:13C&AA:K:N:15N]

DDALL_FLAG_CALIBRATION_18O No

① Set **TYPE_IDENTIFICATION_RESULT** as **pGlyco**.

② Put the identification result file **pGlycoDB-GP-FDR-Pro.txt** here and set **FDR** as **0.01** (default).

③ Set **TYPE_QUANT** as **ReportIon**.

The **RI_MASS_REPORT_ION** could be

126.127726,127.124761,128.134436,129.131471,130.141145,131.138180.

④ Set the Output Folder for saving the quantitation results.

④

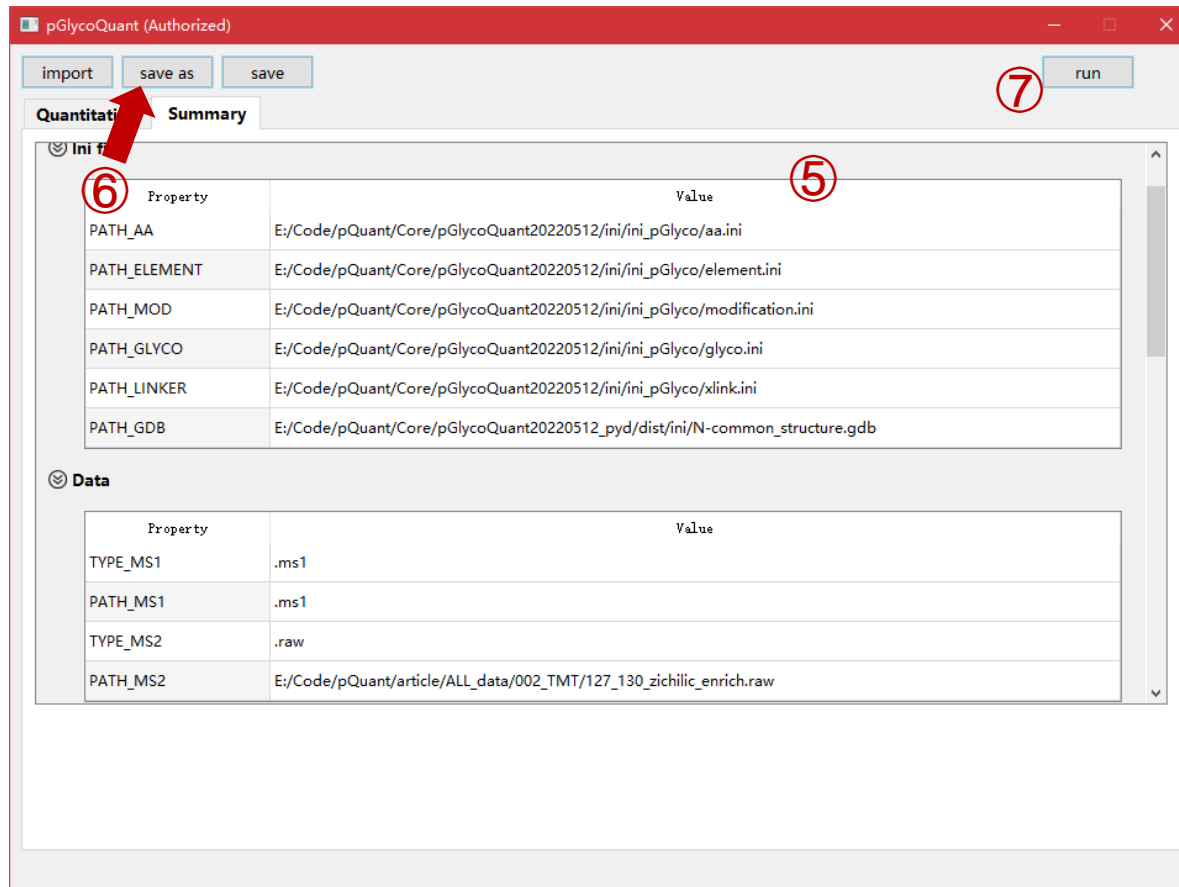
Export

PATH_EXPORT E:/Code/pQuant/article/ALL_data/002_TMT/pQuant2 Browser

FLAG_CREATE_NEW_FOLDER Yes

2 Quantitation with pGlycoQuant

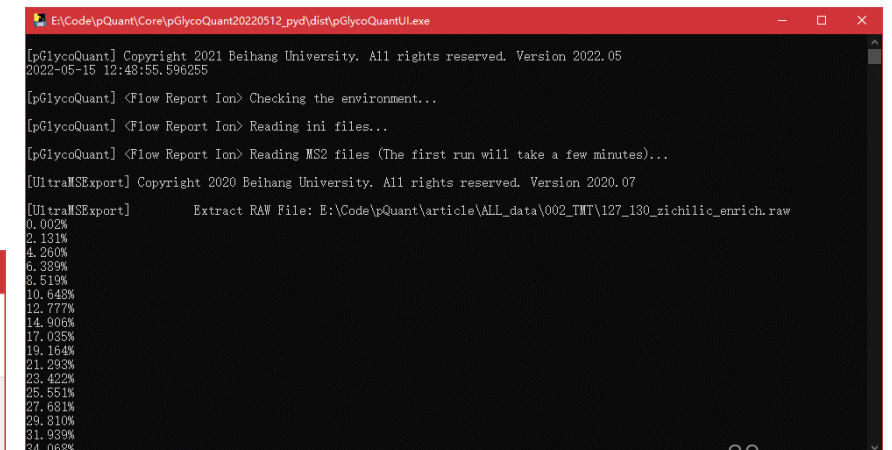
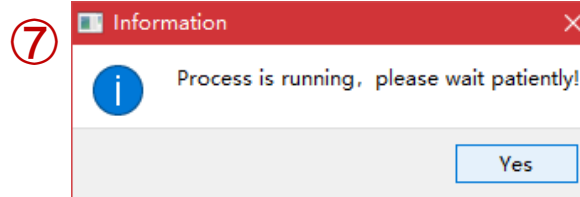
1.3 pGlycoQuant quantitation



⑤ Click **Summary** button and make sure that the **Wrong Option Number** is 0.

⑥ Then click **save as** button to save the config file.

⑦ Click **run** button to start the quantitation, the progress information will be shown in the command-line interface.



2 Quantitation with pGlycoQuant

1.4 quantitation results

①

```
选择E:\Code\pQuant\Core\pGlycoQuant20220512_pyd\dist\pGlycoQuantUI.exe
80.908%
83.037%
85.166%
87.296%
89.425%
91.554%
93.683%
95.812%
97.941%
100.0%

[UltraMSExport]      Extract Result MS1 number:4660, MS2 number:42308
[UltraMSExport] Finished!

[pGlycoQuant]   Creating index file for: E:\Code\pQuant\article\ALL_data\002_TMT\127_130_zichilic_enrich.ms2
[pGlycoQuant] <Flow Report Ion> Reading identification files...
[pGlycoQuant]   Reading E:\Code\pQuant\article\ALL_data\002_TMT\pGlyco\pGlycoDB-GP-FDR-Pro.txt
[pGlycoQuant]   Total number of PSMs: 119
[pGlycoQuant] <Flow Report Ion> Quantifying...
0.840%
100.0%

[pGlycoQuant] <Flow Report Ion> Infering...
[pGlycoQuant] Finished!
```

②

A file explorer window showing a directory containing six files: **cfg.txt**, **pGlycoQuant.modification.list**, **pGlycoQuant.protein.list**, **pGlycoQuant.site.list**, **pGlycoQuant.spectra.info**, and **pGlycoQuant.spectra.list**. A red arrow points from the text "Please open the files with Excel" to the **pGlycoQuant.spectra.info** file.

- ① The completed information.
- ② The quantitation results. Please open the files with Excel.

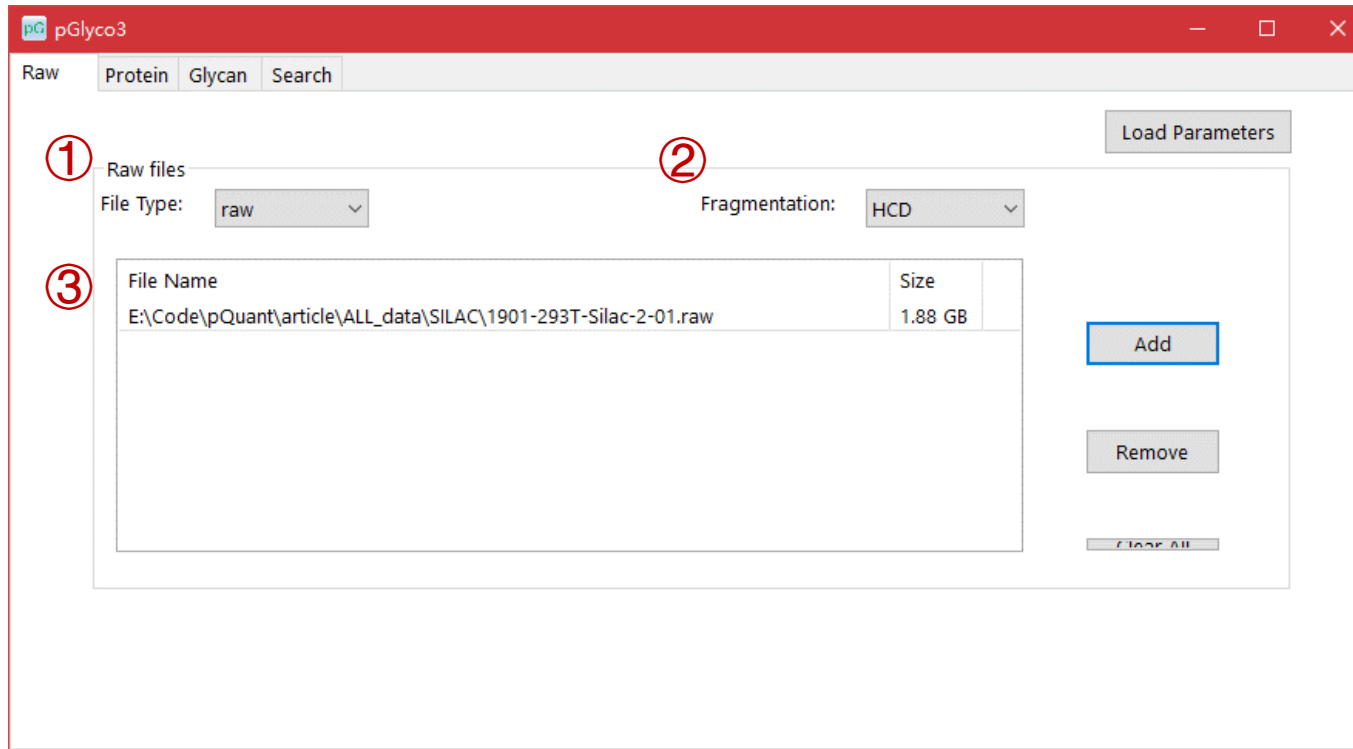
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	GlySpec	PepSpec	RawName	Scan	RT	PrecursorI	PrecursorH	Charge	Rank	Peptide	Mod	PeptideMH	GlycanH	GlycanCon	PlausibleSt	GlyID	GlyFrag
2	127_130_zi127_130_zi127_130_zi			8693	2044.456	2732.231	1366.619	2	1	ILTEEK	1.TMT6ple	1191.699	7.2 0 0 0	H(7/N)2	(N)N(H)H(Q)	11421	0 1 0 0 0
3	127_130_zi127_130_zi127_130_zi			16470	3826.016	3785.689	1262.568	3	1	LGTDPIVA	1.TMT6ple	1759.10	2 0 0 0	H(10/N)2	(N)N(H)H(Q)	10340	0 1 0 0 0
4	127_130_zi127_130_zi127_130_zi			18386	4266.49	4318.853	1080.469	4	1	HNUDTQHI	1.TMT6ple	2940.371	6 2 0 0 0	H(6/N)2	(N)N(H)H(Q)	11463	0 1 0 0 0
5	127_130_zi127_130_zi127_130_zi			18436	4278.043	4156.8	1039.955	4	1	HNUDTQHI	1.TMT6ple	2940.371	5 2 0 0 0	H(5/N)2	(N)N(H)H(Q)	11518	0 1 0 0 0
6	127_130_zi127_130_zi127_130_zi			14834	3473.993	3173.375	1058.463	3	1	FINVQTVS1	1.TMT6ple	1470.79	2 0 0 0	H(8/N)2	(N)N(H)H(Q)	11452	0 1 0 0 0
7	127_130_zi127_130_zi127_130_zi			3762	902.888	2928.189	1464.598	2	1	GVNSACAR	1.TMT6ple	1063.552	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	11358	0 1 0 0 0
8	127_130_zi127_130_zi127_130_zi			32315	7454.437	4185.739	1395.918	3	1	VMSWVD	1.TMT6ple	2321.104	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	14673	0 1 0 0 0
9	127_130_zi127_130_zi127_130_zi			10858	2546.11	3106.41	1036.142	3	1	DAVNITAI	1.TMT6ple	1403.826	8 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	10345	0 1 0 0 0
10	127_130_zi127_130_zi127_130_zi			8700	2046.138	2732.23	911.415	3	1	ILTEEK	1.TMT6ple	1191.699	7 2 0 0 0	H(7/N)2	(N)N(H)H(Q)	10935	0 1 0 0 0
11	127_130_zi127_130_zi127_130_zi			6832	1614.429	2666.108	1333.558	2	1	NLMRSR	1.TMT6ple	963.5243	8 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	10352	0 1 0 0 0
12	127_130_zi127_130_zi127_130_zi			17869	4148.071	3516.618	1758.813	2	1	VFGSQJLT1	1.TMT6ple	1651.978	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	10930	0 1 0 0 0
13	127_130_zi127_130_zi127_130_zi			23445	5414.001	4660.085	1165.777	4	1	TDDEVVQF1	1.TMT6ple	2957.501	8 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	10258	0 1 0 0 0
14	127_130_zi127_130_zi127_130_zi			9928	2332.427	2496.17	832.7281	3	1	SLJCTVK	1.TMT6ple	1279.744	5 2 0 0 0	H(5/N)2	(N)N(H)H(Q)	14652	0 1 0 0 0
15	127_130_zi127_130_zi127_130_zi			26316	6074.413	4163.808	1041.708	4	1	VDLEDFEJH	1.TMT6ple	2087.07	6 4 0 2 0	H(6/N)4(F)G	(N)N(H)H(Q)	11551	0 1 0 0 0
16	127_130_zi127_130_zi127_130_zi			11846	2773.553	3150.329	1575.668	2	1	JYTADYDK	1.TMT6ple	1447.747	8 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	11505	0 1 0 0 0
17	127_130_zi127_130_zi127_130_zi			4857	1157.516	3564.518	1188.844	3	1	YHSQTYGJ	1.TMT6ple	1699.88	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	10342	0 1 0 0 0
18	127_130_zi127_130_zi127_130_zi			8934	2099.288	2944.359	982.1246	3	1	JATLAEQA	1.TMT6ple	1403.826	7 2 0 0 0	H(7/N)2	(N)N(H)H(Q)	11421	0 1 0 0 0
19	127_130_zi127_130_zi127_130_zi			9595	2254.935	2962.329	994.7811	3	1	SLJCTVK	1.TMT6ple	1279.744	8 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	10258	0 1 0 0 0
20	127_130_zi127_130_zi127_130_zi			15997	3717.018	3300.414	1100.809	3	1	ITSWDNA	1.TMT6ple	1597.837	8 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	10352	0 1 0 0 0
21	127_130_zi127_130_zi127_130_zi			18289	4244.351	4642.952	1161.494	4	1	HNUDTQHI	1.TMT6ple	2940.371	6 2 0 0 0	H(6/N)2	(N)N(H)H(Q)	10352	0 1 0 0 0
22	127_130_zi127_130_zi127_130_zi			17753	4121.404	3140.457	1047.491	3	1	VYVLTKE	1.TMT6ple	1437.872	8 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	10352	0 1 0 0 0
23	127_130_zi127_130_zi127_130_zi			19971	4628.824	4257.981	1065.251	4	1	TTLVDMNT1	1.TMT6ple	2555.398	9 2 0 0 0	H(8/N)2	(N)N(H)H(Q)	10345	0 1 0 0 0
24	127_130_zi127_130_zi127_130_zi			8789	2068.808	3268.465	1090.16	3	1	JATLAEQA	1.TMT6ple	1403.826	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	10946	0 1 0 0 0
25	127_130_zi127_130_zi127_130_zi			20062	4650.036	3183.49	1061.835	3	1	VIYPLK	1.TMT6ple	1318.85	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	10930	0 1 0 0 0
26	127_130_zi127_130_zi127_130_zi			24531	5664.887	3703.717	926.6847	4	1	IFIFIQTGIE	1.TMT6ple	1839.078	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	9762	0 1 0 0 0
27	127_130_zi127_130_zi127_130_zi			30710	7087.175	4897.149	1225.043	4	1	IIISPEJMT1	1.TMT6ple	2659.381	7 4 1 0 0	H(7/N)4(A)	(N)N(H)H(Q)	11074	0 1 0 0 0
28	127_130_zi127_130_zi127_130_zi			14847	3453.878	3335.429	1112.481	3	1	FINVQTVS1	1.TMT6ple	1470.79	2 0 0 0	H(9/N)2	(N)N(H)H(Q)	10342	0 1 0 0 0
29	127_130_zi127_130_zi127_130_zi			22649	5233.488	4307.966	1077.747	4	1	AJTTPQPIV1	1.TMT6ple	2070.196	7 4 1 0 0	H(7/N)4(A)	(N)N(H)H(Q)	11074	0 1 0 0 0
30	127_130_zi127_130_zi127_130_zi			20451	4739.856	3689.682	923.1759	4	1	LGTDPIVA	1.TMT6ple	1759.6	4 0 1 0	H(6/N)4(F)G	(N)N(H)H(Q)	11560	0 1 0 0 0
31	127_130_zi127_130_zi127_130_zi			26291	6068.664	4163.807	1388.607	3	1	VDLEDFEJH	1.TMT6ple	2087.07	6 4 0 2 0	H(6/N)4(F)G	(N)N(H)H(Q)	2376	0 1 0 0 0
32	127_130_zi127_130_zi127_130_zi			7845	1846.799	3354.642	1118.886	3	1	GGGIKPPQJ1	1.TMT6ple	1814.11	7 2 0 0 0	H(7/N)2	(N)N(H)H(Q)	11421	0 1 0 0 0
33	127_130_zi127_130_zi127_130_zi			18182	4210.407	3508.608	1200.207	2	1	ELISIASQNA	1.TMT6ple	1722.060	9 2 0 0 0	H(9/N)2	(N)N(H)H(Q)	10227	0 1 0 0 0

pGlyco & pGlycoQuant Manual for SILAC Data



1. Identification with pGlyco

SILAC



- ① Load raw files.
- ② Set the **Fragmentation** as **HCD**.
- ③ Add the **SILAC** raw file.



1. Identification with pGlyco

The screenshot shows the pGlyco3 software interface with the following settings and annotations:

- Annotation 1:** Points to the **Fasta:** field containing the path `E:\Code\pQuant\article\ALL_data\SILAC\Human-H.sapiens-SP-1808_N2J_STC.fasta`.
- Annotation 2:** Points to the **Enzyme** section where **Name:** is set to `Trypsin`, **Digestion:** is set to `specific`, and **Max Miss Cleavage:** is set to `2`.
- Annotation 3:** A red box highlights the **Fixed Protein Modifications** section, which contains `Carbamidomethyl[C]`. Below it, the **Variable Protein Modifications** section lists `Oxidation[M]`, `Acetyl[ProteinN-term]` (highlighted in blue), `Label_13C(6)[K]`, and `Label_13C(6)[R]`.
- Annotation 4:** Points to the bottom filter section where **Max Var Mod on Peptide:** is `2`, **Peptide Length:** is from `6` to `40`, and **Peptide Mass:** is from `600` to `4000`.

Additional interface elements include a **Modification List** on the right with various labeled modifications (e.g., `Label_13C(4)15N(1)[D]`), **<<** and **>>** buttons for moving items between lists, and an **Update** button.

- ① Set the fasta database (The file could be downloaded from <https://github.com/expellir-arma/pGlycoQuant/>).
- ② Set the trypsin enzyme.
- ③ Set the fixed modification as Carbamidomethyl on Cys site. Set the variable modification as Acetyl on Protein N-Term, Oxidation on Met site, **Label_13C(6)[K] on Lys site and Label_13C(6)[R] on Arg site.**
- ④ Set the filter information.

1. Identification with pGlyco

SILAC

The screenshot shows the pGlyco3 software window with a red title bar. It has four tabs: 'Raw', 'Protein', 'Glycan', and 'Search'. The 'Glycan' tab is selected. At the top, there are two dropdown menus: 'Glycan DB:' set to 'pGlyco-N-Human.gdb' (annotated with a red circle 1) and 'Glycan Type:' set to 'N-Glycan' (annotated with a red circle 2). To the right of these is a 'Convert GlycoWorkbench' button. Below these are two sections: 'Fixed Glycan Modifications' and 'Variable Glycan Modifications'. The 'Fixed' section has an empty list box, '<<' and '>>' buttons, and a 'Glyco:' dropdown set to 'N' with a 'Modified as:' dropdown. The 'Variable' section (annotated with a red circle 3) has a list box containing 'H~pH', '<<' and '>>' buttons, a 'Glyco:' dropdown set to 'H', and a 'Modified as:' dropdown set to 'pH'. At the bottom, there are two input fields: 'Max Var Mod on Glycan:' with the value '1' and 'Max Number of (Modified) Glycans to Search:' with the value '100000'.

① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.

② Set the **Glycan Type** as **Glycan**.

③ Set the Glycan modification information.

1. Identification with pGlyco

The screenshot shows the pGlyco3 software interface with the following settings and annotations:

- ①** Precursor Tolerance: ± 4 ppm
- ②** Fragment Tolerance: ± 20 ppm
- ③** Number of Processes: 5
- ④** Glycopeptide FDR: 0.01
- ☐ Percolator
- ☐ FMM for Peptide FDR
- ☒ pGlycoSite: Localized Glycans Must Be in GDB
- pGlycoNovo**
 - ☐ Run pGlycoNovo
 - Glyco: H Max: 20
 - Glyco: N Max: 7
 - Glyco: F Max: 5
 - Glyco: A Max: 4
 - Glyco: (empty) Max: (empty)
 - Glyco: (empty) Max: (empty)
 - Glyco: (empty) Max: (empty)
 - Glyco: (empty) Max: (empty)
- Allow Max Glyco Gap: 3
- ⑤** Output Folder: E:\Code\pQuant\article\ALL_data\SILAC\pGlyco
- ⑥** Run, Save, Stop buttons

- ① Set the **Precursor Tolerance** as ± 4 ppm.
- ② Set the **Fragment Tolerance** as ± 20 ppm.
- ③ Set the **Number of Processes** according to your PC.
- ④ Set the **Glycopeptide FDR** as **0.01**.
- ⑤ Set the **Output Folder** for saving the identification results.
- ⑥ Click **Save** and **Run** buttons, the progress information will be shown in the command-line interface.

```
C:\WINDOWS\system32\cmd.exe
E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615>cd bin
E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlyco3GUI.exe
Already registered!
E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\pGlyco.cfg
E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\pGlyco.cfg
pGlyco3 Starting task...
Process ID=21796: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>rem *****
*****
Process ID=21796: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>parse_raw.exe "E:\Code\pQuant\article\ALL_d
ata\SILAC\pGlyco\process1\pGlyco.cfg
Process ID=21796: pGlyco *****
Process ID=21796: pParse (x64) from pFind Studio
Process ID=21796: Email : pfind@ict.ac.cn
Process ID=21796: Website: http://pfind.ict.ac.cn
Process ID=21796: *****
Process ID=21796: The license will expire in 2100-1-1
Process ID=21796: [pParse] C:\INFO: - pParse writes logs in E:\Code\pQuant\article\ALL_data\SILAC\pParsePlusLog.txt
Process ID=21796: [pParse] C:\INFO: - BEGIN PARAMETERS
Process ID=21796: [pParse] C:\INFO: - 01: check_activationcenter = 1
Process ID=21796: [pParse] C:\INFO: - 02: co-elute = 1
Process ID=21796: [pParse] C:\INFO: - 03: cut_similar_mono = 1
Process ID=21796: [pParse] C:\INFO: - 04: datanum = 1
Process ID=21796: [pParse] C:\INFO: - 05: datapath1 = E:\Code\pQuant\article\ALL_data\SILAC\1901-293T-Silac-2-01.ms1
Process ID=21796: [pParse] C:\INFO: - 06: delete_msn = 0
Process ID=21796: [pParse] C:\INFO: - 07: dia_mode = 0
Process ID=21796: [pParse] C:\INFO: - 08: dia_mode_to_filter_by_selectedMS2ScanNoSet = 1
Process ID=21796: [pParse] C:\INFO: - 09: input_format = ms1
Process ID=21796: [pParse] C:\INFO: - 10: intensity = 1
```

1. Identification with pGlyco

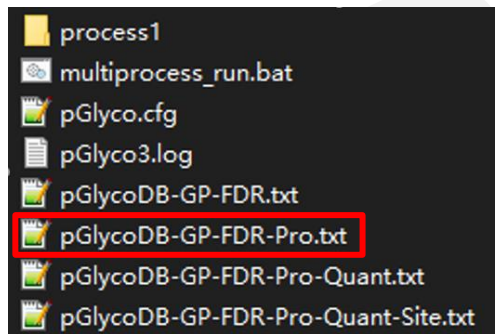
①

```
C:\WINDOWS\system32\cmd.exe
Process ID=21796: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>gpPercolator.exe -p "E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlyco.cfg"
Process ID=21796: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlycoFDR.exe -p "E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlyco.cfg"
Process ID=21796: E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlycoDB-GP-Raw1-FDR.txt
Process ID=21796: 1009 GPSMs at 1.0% FDR
Process ID=21796: E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlycoDB-GP-Raw1-FDR-noFiltered.txt
Process ID=21796: 6608 GPSMs at 100.0% FDR
Process ID=21796: merge into E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlycoDB-GP-FDR.txt
Process ID=21796: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlycoProInfer.exe -p "E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlyco.cfg"
Process ID=21796: Reading pGlyco results ...
Process ID=21796: Inferring proteins ...
Process ID=21796: End inference
Process ID=21796: E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlycoDB-GP-FDR-Pro.txt
Process ID=21796: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>XIC.exe -p "E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlyco.cfg"
Process ID=21796: [XIC] Smoothing window = 21
Process ID=21796: [XIC] Smoothing method = savgol_filter
Process ID=21796: [XIC] Loading pGlyco results ...
Process ID=21796: [XIC] RT window is [-120.0, +120.0] seconds
Process ID=21796: [XIC] Indexing e:\code\pquant\article\all_data\silac\1901-293t-silac-2-01.pfl
Process ID=21796: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin>pGlycoSite.exe "E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\process1\pGlyco.cfg"
Process ID=21796: Already registered!
Process ID=21796: [pGlycoSite] Glycosylation site localization finished!
[pGlyco] All results are merged!
[pGlyco] Running time = 40.3 minutes.
[pGlyco] Task completed!
```

① The completed information in the command-line interface.

② The identification results.

②



2 Quantitation with pGlycoQuant

SILAC

1.3 pGlycoQuant quantitation

①

②

① Ensure that the ini file paths are valid.

② Set the **TYPE_MS1** as **.raw** and fill the raw files into the **PATH_MS1** blank.

2 Quantitation with pGlycoQuant

SILAC

1.3 pGlycoQuant quantitation

pGlycoQuant (Authorized)

import save as save run

Quantitation Summary

Identification Result

① TYPE_IDENTIFICATION_RESULT: pGlyco

② PATH_IDENTIFICATION_RESULT: E:/Code/pQuant/article/ALL_data/003_SILAC/pQuant3/pGlycoDB-GP-FDR-Pro-H_new.txt
E:/Code/pQuant/article/ALL_data/003_SILAC/pQuant3/pGlycoDB-GP-FDR-Pro-L_new.txt

THRESHOLD_FDR: 0.010

FLAG_MIR_GLYCO: No

Quantitation

③ TYPE_QUANT: DDA Labeling

RI_PPM_HALF_WIN_ACCURACY_PEAK: 1000.00

RI_MASS_REPORT_ION: 127.11, 130.11

DDALL_RT_HALF_WIN_IN_MIN: 2.00

DDALL_PPM_HALF_WIN_ACCURACY_PEAK: 20.00

DDALL_LABEL_INFO: 2|MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|AA:K:C:13C&AA:R:C:13C&MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|

DDALL_FLAG_CALIBRATION_18O: No

Export

④ PATH_EXPORT: E:/Code/pQuant/article/ALL_data/SILAC/pQuant

FLAG_CREATE_NEW_FOLDER: No

① Set **TYPE_IDENTIFICATION_RESULT** as **pGlyco**.

② Put identification results in **PATH_IDENTIFICATION_RESULT** blank and set **FDR** as **0.01**.

③ Set **TYPE_QUANT** as **DDA Labeling**.

Set **DDALL_RT_HALF_WIN_IN_MIN** as **2.00** minutes (default) and set **DDALL_PPM_HALF_WIN_ACCURACY_PEAK** as **20.00** ppm (default).

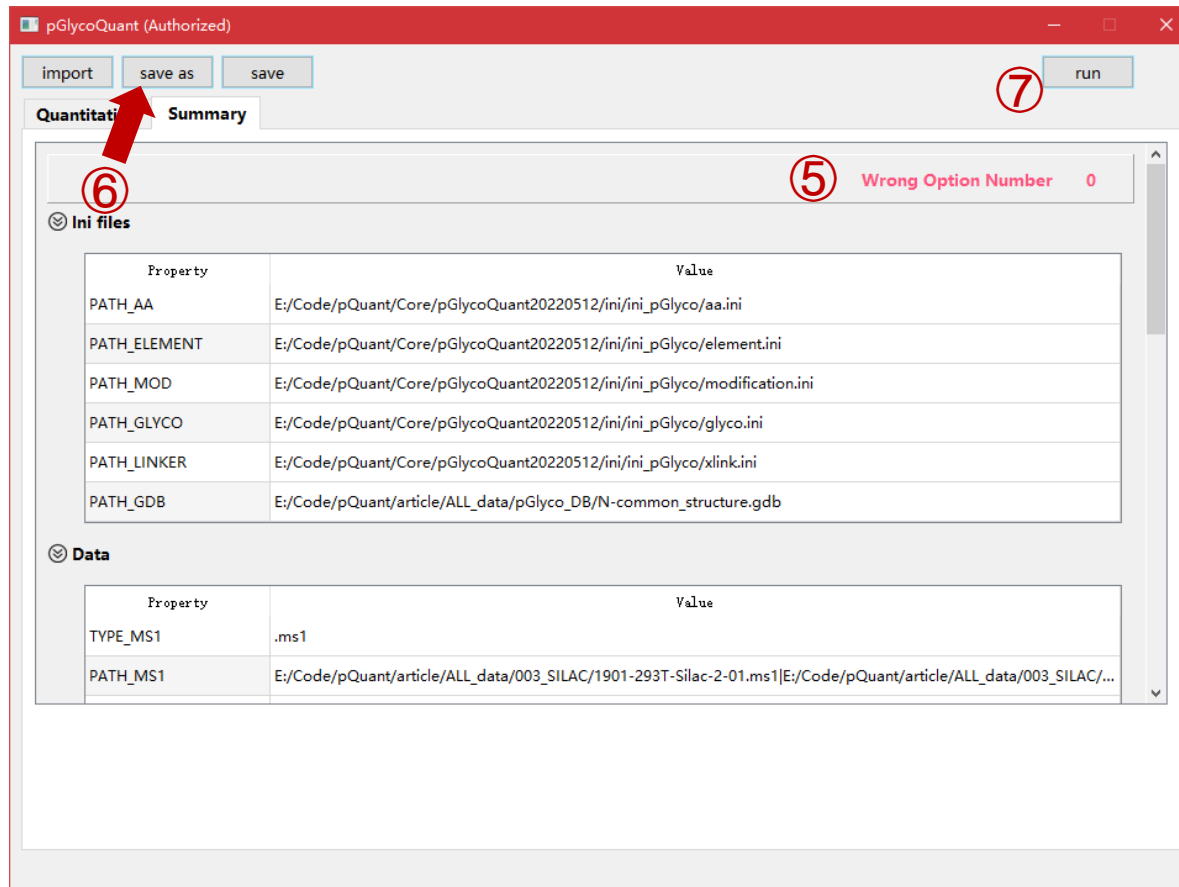
The DDALL_LABEL_INFO could be **2|MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|AA:K:C:13C&AA:R:C:13C&MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|**.

④ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

SILAC

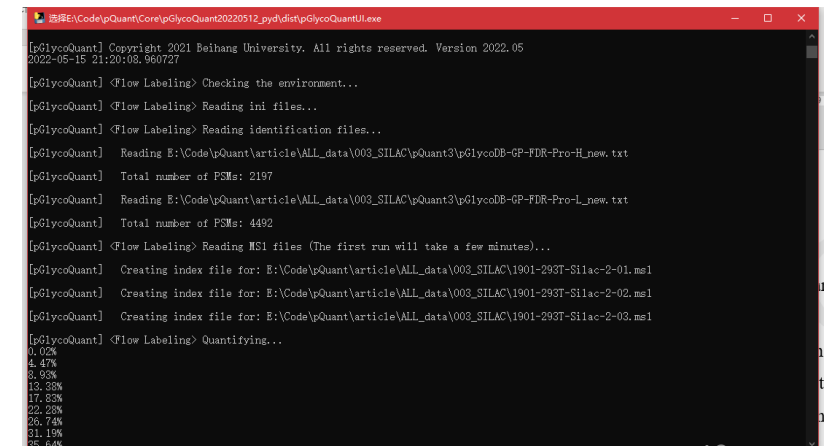
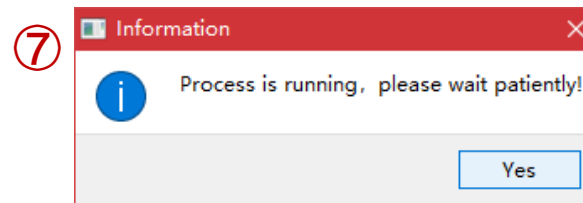
1.3 pGlycoQuant quantitation



⑤ Click **Summary** button and make sure that the **Wrong Option Number** is 0.

⑥ Then click **save as** button to save the config file.

⑦ Click **run** button to start the quantitation, the progress information will be shown in the command-line interface.



2 Quantitation with pGlycoQuant

1.4 quantitation results

①

```
E:\Code\pQuant\Core\pGlycoQuant20220512_py\dist\pGlycoQuantUI.exe

[pGlycoQuant] Creating index file for: E:\Code\pQuant\article\ALL_data\003_SILAC\1901-293T-Silac-2-01.ms1
[pGlycoQuant] Creating index file for: E:\Code\pQuant\article\ALL_data\003_SILAC\1901-293T-Silac-2-02.ms1
[pGlycoQuant] Creating index file for: E:\Code\pQuant\article\ALL_data\003_SILAC\1901-293T-Silac-2-03.ms1

[pGlycoQuant] <Flow Labeling> Quantifying...
0.02%
4.47%
8.93%
13.38%
17.83%
22.28%
26.74%
31.19%
35.64%
40.09%
44.55%
49.00%
53.45%
57.90%
62.36%
66.81%
71.26%
75.71%
80.16%
84.62%
89.07%
93.52%
97.97%
100.0%

[pGlycoQuant] <Flow Labeling> Inferring...
[pGlycoQuant] Finished!
```

②

- cfg.txt
- pGlycoQuant.modification.list
- pGlycoQuant.protein.list
- pGlycoQuant.site.list
- pGlycoQuant.spectra.info
- pGlycoQuant.spectra.list

- ① The completed information.
- ② The quantitation results. Please open the files with Excel.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q						
1	GlySpec	PepSpec	RawName	Scan	RT	Precursor	PrecursorCh	Charge	Rank	Peptide	Mod	PeptideMH	Glycan	Con	PlausibleSt	GlyID	GlyFrag						
2	1901-293T1901-293T1901-293T	37314	10074.55	2779.288	927.1007	3	1	TJSTFPVQAI14	Label.1	1578.858	4	2	0	1	0	H(4)N(2)F(1)N(F)N(H)H	29	0	1	0	0	0	
3	1901-293T1901-293T1901-293T	19060	5153.299	2477.076	1239.042	2	1	JISQVLEK	8	Label.13	936.5456	7	2	0	0	0	H(7)N(2)	(N)N(H)H	114	0	1	0	0
4	1901-293T1901-293T1901-293T	31846	8629.107	2409.123	1205.065	2	1	NNVTIILTH	11	Label.1	1192.699	5	2	0	0	0	H(5)N(2)	(N)N(H)H	31	0	1	0	0
5	1901-293T1901-293T1901-293T	3029	819.1295	2381.977	1191.492	2	1	SLSISTAR	8	Label.13	841.4477	7	2	0	0	0	H(7)N(2)	(N)N(H)H	114	0	1	0	0
6	1901-293T1901-293T1901-293T	47502	12815.46	3288.455	1096.823	3	1	LHLAGGC16	Label.1	1747.926	7	2	0	0	0	0	H(7)N(2)	(N)N(H)H	114	0	1	0	0
7	1901-293T1901-293T1901-293T	9991	2702.409	3173.289	794.0776	4	1	YHYGTFEE11	Label.1	1470.705	8	2	0	0	0	0	H(8)N(2)	(N)N(H)H	210	0	1	0	0
8	1901-293T1901-293T1901-293T	9773	2643.202	2860.077	1430.542	2	1	JYTADYDK	8	Label.13	995.4412	9	2	0	0	0	H(9)N(2)	(N)N(H)H	344	0	1	0	0
9	1901-293T1901-293T1901-293T	24506	6621.231	2912.326	971.4468	3	1	LEIITGTGY15	Label.1	1695.901	5	2	0	0	0	0	H(5)N(2)	(N)N(H)H	31	0	1	0	0
10	1901-293T1901-293T1901-293T	3142	849.1516	2381.977	1191.492	2	1	SLSISTAR	8	Label.13	841.4477	7	2	0	0	0	H(7)N(2)	(N)N(H)H	121	0	1	0	0
11	1901-293T1901-293T1901-293T	17344	4689.178	3112.367	1038.127	3	1	WTGHJVTI11	Label.1	1302.701	4	5	0	1	0	0	H(4)N(5)F(1)N(F)N(H)H	291	0	1	0	0	
12	1901-293T1901-293T1901-293T	16686	4511.896	2908.226	970.08	3	1	HTNMLTCT10	Label.1	1205.64	8	2	0	0	0	0	H(8)N(2)	(N)N(H)H	201	0	1	0	0
13	1901-293T1901-293T1901-293T	7274	1968.482	2492.051	1246.529	2	1	JATLAEQA9	Label.13	951.5201	7	2	0	0	0	0	H(7)N(2)	(N)N(H)H	114	0	1	0	0
14	1901-293T1901-293T1901-293T	29531	7976.149	2683.162	1342.084	2	1	TCDWLPKF2	Carbamid	1806.837	2	2	0	1	0	0	H(2)N(2)F(1)N(F)N(H)H	8	0	1	0	0	
15	1901-293T1901-293T1901-293T	25402	6863.698	2699.153	900.3893	3	1	TCDWLPKF2	Carbamid	1822.832	2	2	0	1	0	0	H(2)N(2)F(1)N(F)N(H)H	8	0	1	0	0	
16	1901-293T1901-293T1901-293T	24518	6624.43	2699.155	900.3898	3	1	TCDWLPKF2	Carbamid	1822.832	2	2	0	1	0	0	H(2)N(2)F(1)N(F)N(H)H	8	0	1	0	0	
17	1901-293T1901-293T1901-293T	15093	4080.771	2250.057	750.6905	3	1	LKPLFK	2	Label.13	871.5803	6	2	0	0	0	H(6)N(2)	(N)N(H)H	61	0	1	0	0
18	1901-293T1901-293T1901-293T	20275	5478.06	2657.177	1329.092	2	1	ALSPJSTIS13	Label.1	1278.7	6	2	0	0	0	0	H(6)N(2)	(N)N(H)H	60	0	1	0	0
19	1901-293T1901-293T1901-293T	32846	8871.816	2808.224	1404.616	2	1	AGPIGTLP14	Label.1	1429.742	6	2	0	0	0	0	H(6)N(2)	(N)N(H)H	60	0	1	0	0
20	1901-293T1901-293T1901-293T	27224	7355.377	2941.278	981.0974	3	1	JHSIFLADI13	Label.1	1562.802	6	2	0	0	0	0	H(6)N(2)	(N)N(H)H	61	0	1	0	0
21	1901-293T1901-293T1901-293T	25558	6932.579	3016.586	754.902	4	1	TAAQVQG19	Label.1	2610.425	0	2	0	0	0	0	N(2)	(N)N	2	0	1	0	0
22	1901-293T1901-293T1901-293T	14954	4042.744	2523.057	1262.032	2	1	JITSWK	6	Label.13	754.4189	5	4	0	1	0	H(5)N(4)F(1)N(F)N(H)H	256	0	1	0	0	
23	1901-293T1901-293T1901-293T	31060	8390.148	4213.963	1054.246	4	1	AGKPSAAV3	Label.13	2835.489	6	2	0	0	0	0	H(6)N(2)	(N)N(H)H	60	0	1	0	0
24	1901-293T1901-293T1901-293T	3088	834.6995	1799.753	900.3803	2	1	YETTK	6	Label.13	761.3771	3	2	0	1	0	H(3)N(2)F(1)N(F)N(H)H	14	0	1	0	0	
25	1901-293T1901-293T1901-293T	40354	10891.39	3312.499	828.8802	4	1	TJSTFPVQAI14	Label.1	2274.122	3	2	0	1	0	0	H(3)N(2)F(1)N(F)N(H)H	15	0	1	0	0	
26	1901-293T1901-293T1901-293T	13643	3689.379	2598.061	1299.534	2	1	LIITCESSK	5	Carbamid	1057.529	7	2	0	0	0	H(7)N(2)	(N)N(H)H	114	0	1	0	0
27	1901-293T1901-293T1901-293T	12126	3277.629	2654.102	1327.555	2	1	DAVNITAI9	Label.13	951.5201	8	2	0	0	0	0	H(8)N(2)	(N)N(H)H	203	0	1	0	0
28	1901-293T1901-293T1901-293T	22204	5996.889	2950.231	1475.619	2	1	FINVQTVS16	Label.1	1247.647	8	2	0	0	0	0	H(8)N(2)	(N)N(H)H	215	0	1	0	0
29	1901-293T1901-293T1901-293T	31138	8411.065	2649.111	883.7086	3	1	DUETIHYV11	Label.1	1432.687	5	2	0	0	0	0	H(5)N(2)	(N)N(H)H	31	0	1	0	0
30	1901-293T1901-293T1901-293T	9970	2696.716	2664.093	1332.55	2	1	GPTJJTCV7	Carbamid	1123.562	7	2	0	0	0	0	H(7)N(2)	(N)N(H)H	114	0	1	0	0
31	1901-293T1901-293T1901-293T	19506	5272.748	2209.938	1105.473	2	1	VJETEMDI10	Label.1	1155.566	4	2	0	0	0	0	H(4)N(2)	(N)N(H)H	17	0	1	0	0
32	1901-293T1901-293T1901-293T	13061	3532.085	2591.131	864.3817	3	1	NHTASIL10	Label.1	1146.596	3	4	0	1	0	0	H(3)N(4)F(1)N(F)N(H)H	87	0	1	0	0	
33	1901-293T1901-293T1901-293T	12640	3417.62	1042.788	072.308	0	1	JETMNEK	7	Label.13	880.418	1	2	0	0	0	H(4)N(2)	(N)N(H)H	10	0	1	0	0

Notes for Choosing the Input File for pGlycoQuant



Choose the related type of identification result for quantitation

☺ Identification Result

TYPE_IDENTIFICATION_RESULT pGlyco

PATH_IDENTIFICATION_RESULT

THRESHOLD_FDR

Add

Delete

Clear

pFind

pGlyco

Byonic

PD

pGlycoOLD

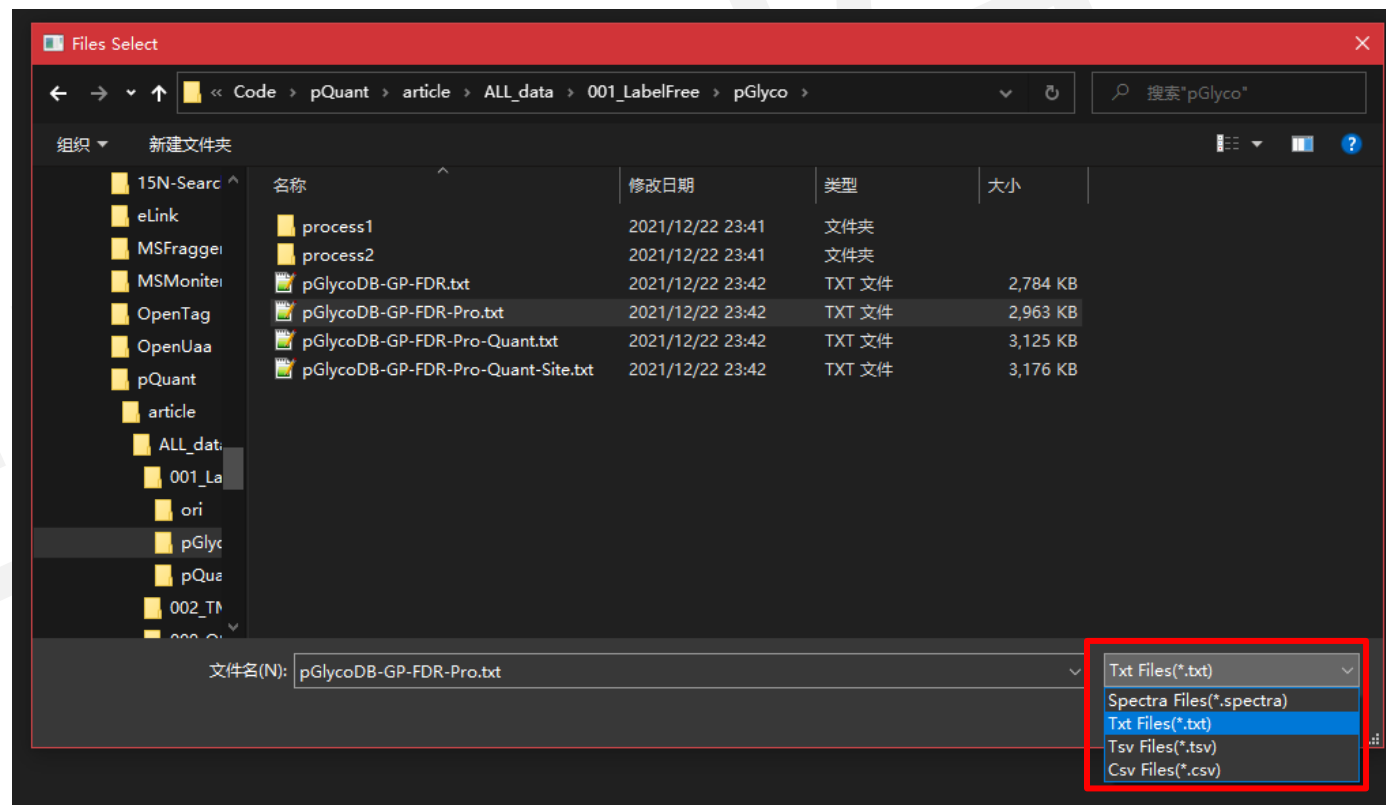
pLink

MSFragger

MSFragger glyco-N

pGlyco results

process1	
multiprocess_run.bat	1 KB
pGlyco.cfg	2 KB
pGlyco3.log	6 KB
pGlycoDB-GP-FDR.txt	487 KB
pGlycoDB-GP-FDR-Pro.txt	520 KB
pGlycoDB-GP-FDR-Pro-Quant.txt	551 KB
pGlycoDB-GP-FDR-Pro-Quant-Site.txt	561 KB



Choose the related type of identification result for quantitation

☺ Identification Result

TYPE_IDENTIFICATION_RESULT MSFragger glyco-N

PATH_IDENTIFICATION_RESULT

THRESHOLD_FDR

Add

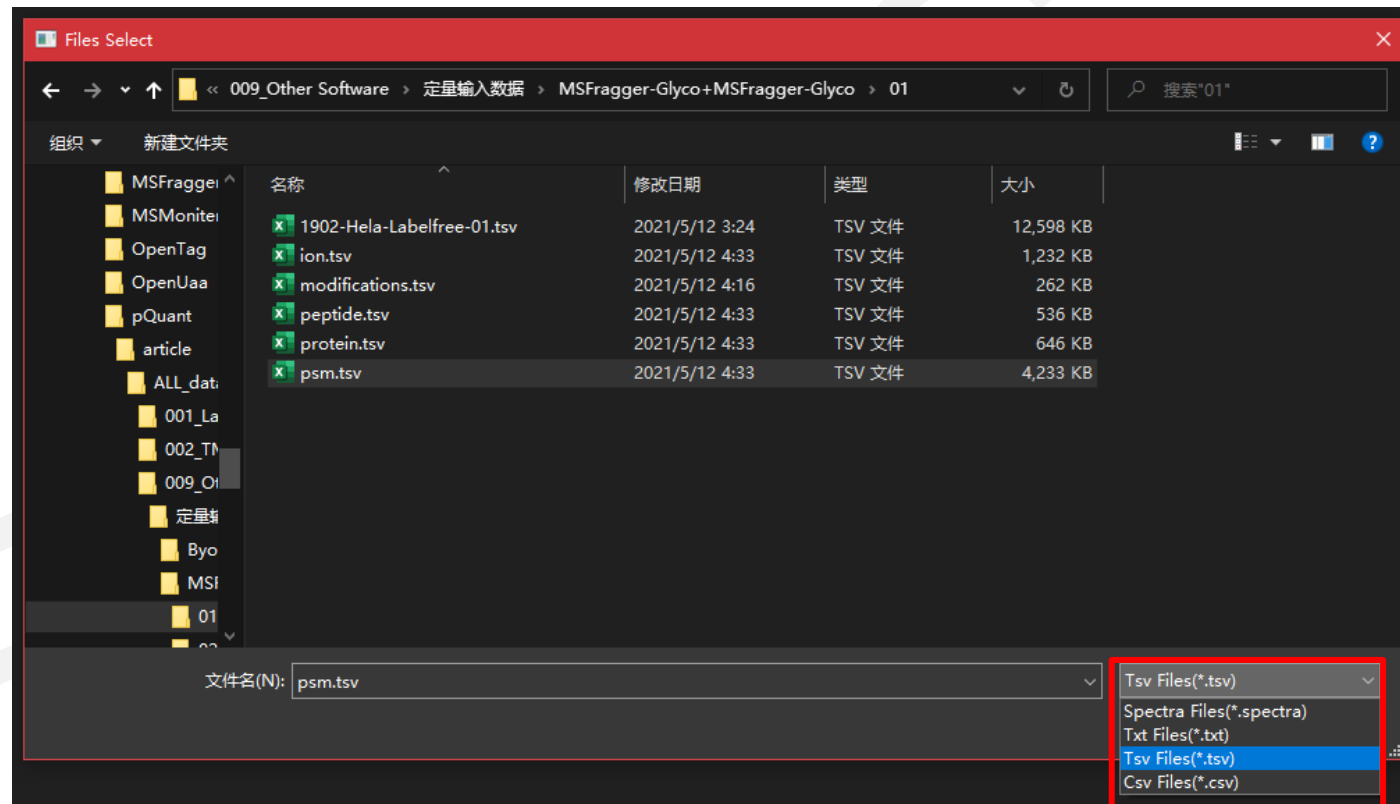
Delete

Clear

MSFragger glyco-N

MSFragger results

1902-Hela-Labelfree-01.pepXML	50,753 KB
1902-Hela-Labelfree-01.tsv	12,598 KB
1902-Hela-Labelfree-01_model.png	72 KB
1902-Hela-Labelfree-01_quant.csv	10,217 KB
delta-mass.html	300 KB
filter.log	3 KB
interact.pep.xml	34,841 KB
ion.tsv	1,232 KB
modifications.tsv	262 KB
peptide.tsv	536 KB
protein.fas	2,212 KB
protein.tsv	646 KB
psm.tsv	4,233 KB



Choose the related type of identification result for quantitation

☑ Identification Result

TYPE_IDENTIFICATION_RESULT

Byonic

pFind

pGlyco

Byonic

PD

pGlycoOLD

pLink

MSFragger

MSFragger glyco-N

Add

Delete

Clear

PATH_IDENTIFICATION_RESULT

THRESHOLD_FDR

Byonic results

1901-293T-SILAC-Byologic-K0R0.txt	933 KB
1901-293T-SILAC-Byologic-K0R0.xlsx	406 KB
1901-293T-SILAC-Byologic-K6R6.txt	360 KB
1901-293T-SILAC-Byologic-K6R6.xlsx	163 KB
1901-293T-SILAC-Byonic-K0R0-1.spectra.txt	3,050 KB
1901-293T-SILAC-Byonic-K0R0-1.xlsx	1,640 KB
1901-293T-SILAC-Byonic-K0R0-2.spectra.txt	3,161 KB
1901-293T-SILAC-Byonic-K0R0-2.xlsx	1,701 KB
1901-293T-SILAC-Byonic-K0R0-3.spectra.txt	3,115 KB
1901-293T-SILAC-Byonic-K0R0-3.xlsx	1,668 KB
1901-293T-SILAC-Byonic-K6R6-1.spectra.txt	1,063 KB
1901-293T-SILAC-Byonic-K6R6-1.xlsx	683 KB
1901-293T-SILAC-Byonic-K6R6-2.spectra.txt	1,151 KB
1901-293T-SILAC-Byonic-K6R6-2.xlsx	732 KB
1901-293T-SILAC-Byonic-K6R6-3.spectra.txt	1,159 KB
1901-293T-SILAC-Byonic-K6R6-3.xlsx	734 KB

Files Select

<< article > ALL_data > 009_Other Software > 定量输入数据 > Byonic_Byologic

搜索"Byonic_Byologic"

组织

新建文件夹

15N-Search

eLink

MSFragger

MSMonitor

OpenTag

OpenUaa

pQuant

article

ALL_data

001_La

002_TM

009_Oi

定量输入数据

Byo

名称	修改日期	类型	大小
1901-293T-SILAC-Byologic-K0R0.txt	2021/4/20 17:50	TXT 文件	933 KB
1901-293T-SILAC-Byologic-K6R6.txt	2021/4/20 18:07	TXT 文件	360 KB
1901-293T-SILAC-Byonic-K0R0-1.spectra.txt	2021/4/19 21:29	TXT 文件	3,050 KB
1901-293T-SILAC-Byonic-K0R0-2.spectra.txt	2021/4/19 21:29	TXT 文件	3,161 KB
1901-293T-SILAC-Byonic-K0R0-3.spectra.txt	2021/4/19 21:32	TXT 文件	3,115 KB
1901-293T-SILAC-Byonic-K6R6-1.spectra.txt	2021/4/19 21:32	TXT 文件	1,063 KB
1901-293T-SILAC-Byonic-K6R6-2.spectra.txt	2021/4/19 21:32	TXT 文件	1,151 KB
1901-293T-SILAC-Byonic-K6R6-3.spectra.txt	2021/4/19 21:32	TXT 文件	1,159 KB

文件名(N): 1901-293T-SILAC-Byonic-K0R0-1.spectra.txt

Txt Files(*.txt)

Spectra Files(*.spectra)

Txt Files(*.txt)

Tsv Files(*.tsv)

Csv Files(*.csv)

Thanks!

