

pGlycoQuant Manual

version.2021.12



Version: pGlycoQuant_V1.1

Release Date: 2021.11.19

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 Manual for SILAC Data](#)
- [Notes for Choosing the Input File for pGlycoQuant](#)



pGlyco & pGlycoQuant

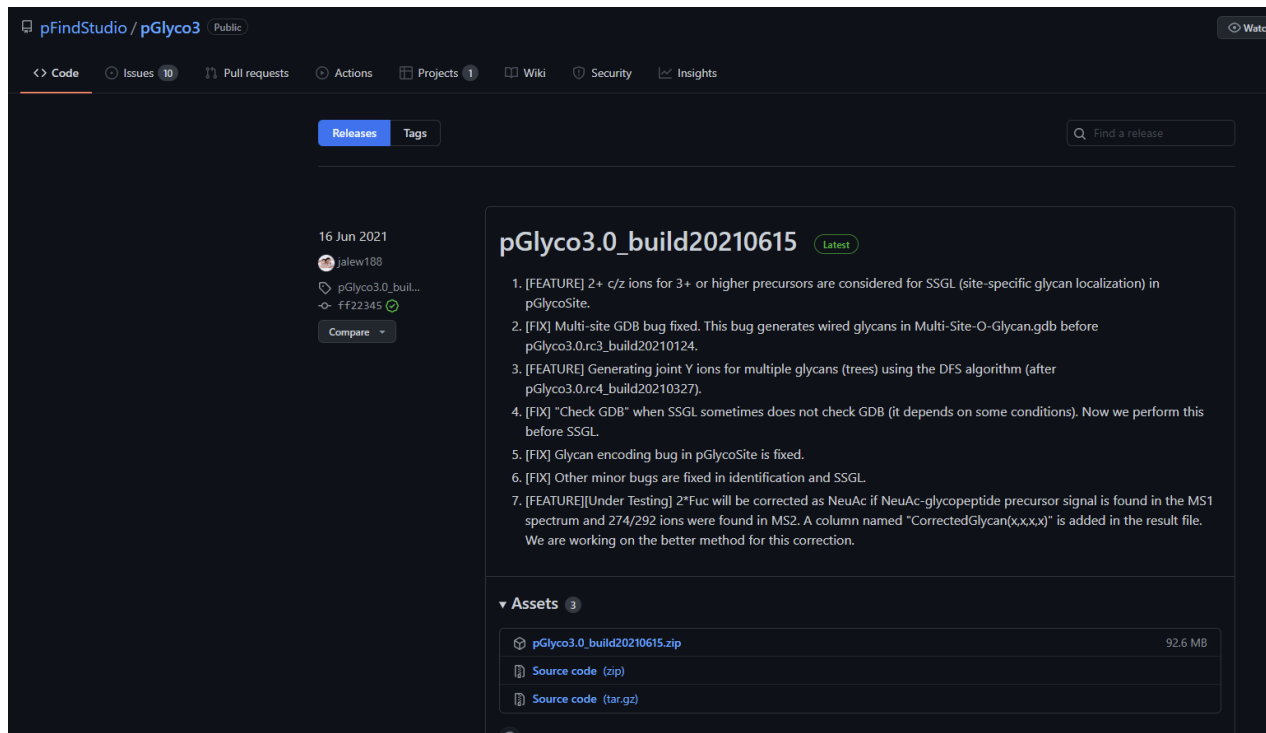
Download and Regist



1 Identification with pGlyco

1.1 pGlyco download

①



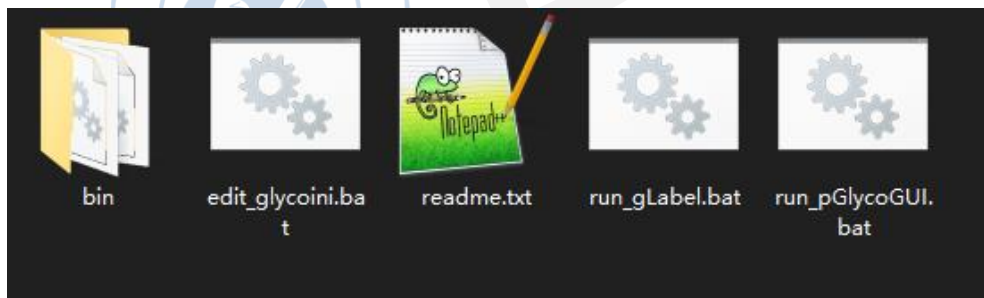
① Login

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

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

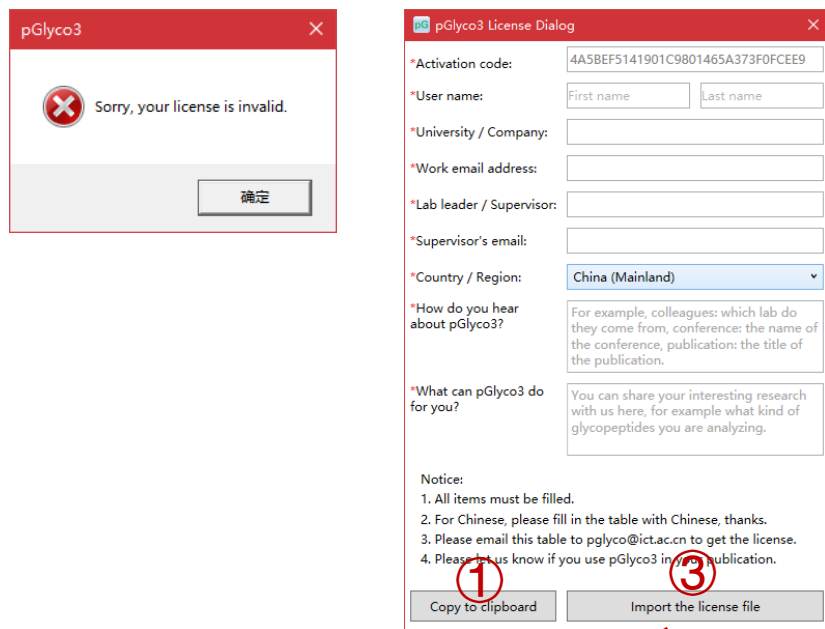
② The unzipped pGlyco files.

②

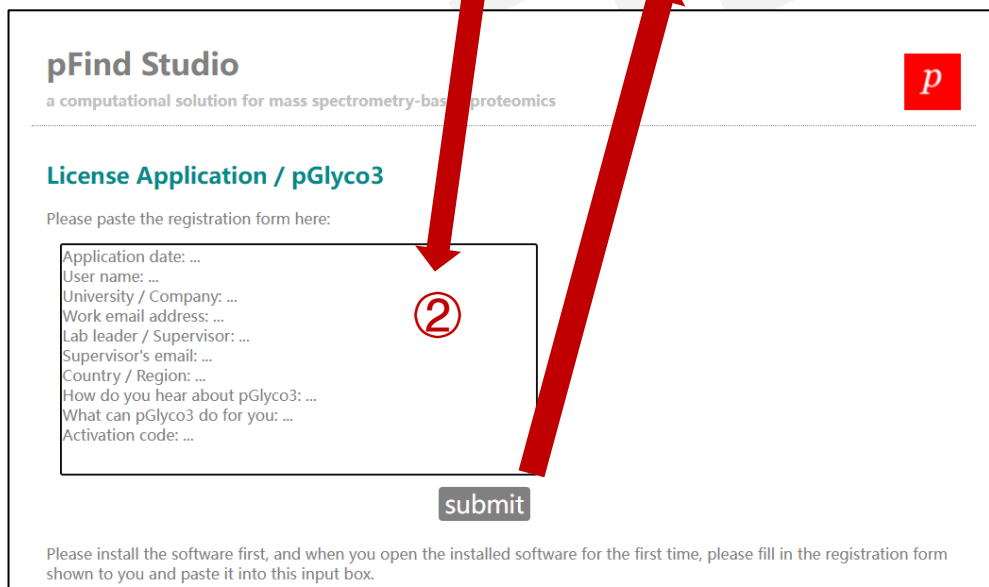


1 Identification with pGlyco

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' interface, specifically the 'License Application / pGlyco3' section. It instructs the user to 'Please paste the registration form here:'. Below this is a large text area containing the registration form data, which is a copy of the information from the 'pGlyco3 License Dialog'. The form includes fields for 'Application date', 'User name', 'University / Company', 'Work email address', 'Lab leader / Supervisor', 'Supervisor's email', 'Country / Region', 'How do you hear about pGlyco3', 'What can pGlyco3 do for you', and 'Activation code'. A red arrow labeled with a circled 2 points from the 'Copy to clipboard' button in the license 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 license 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.

1 pGlycoQuant download

①

Releases / pGlycoQuant_V1.1_20211119

pGlycoQuant_V1.1 Latest

expelliir-arma released this 19 Nov 2021 · 2 commits to main since this release · pGlycoQuant_... · 63ed4fa

Here, we report pGlycoQuant, a generic software tool for accurate and convenient quantitative intact glycopeptide analysis, supporting both primary and tandem mass spectrometry quantitation for multiple quantitative strategies. pGlycoQuant enables intact glycopeptide quantitation with minuscule missing value via a deep residual network and includes a quantitative bioinformatics analysis module. At present, pFind, pGlyco, Byonic and MSFragger software glycosylation identification results can be used for quantification by pGlycoQuant.

Version: pGlycoQuant_V1.1

Release Date: 2021.11.19

Computer configuration

RAM: 16G or higher is recommended

ROM: for one raw data (1G) 5G or higher is recommended

OS: Windows10 or higher

Other: MSFileReader 3.0 Sp1 or higher is needed

Description

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

GUI Operation Usage

Please read Manual_version.2011.11.pdf to learn the usage of pGlycoQuant.

The dataset used for demo also can be found in Manual_version.2011.11.pdf.

Other notes

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 scores>200 and absolute value of LogProb >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_MSFrager directory.

Cite us

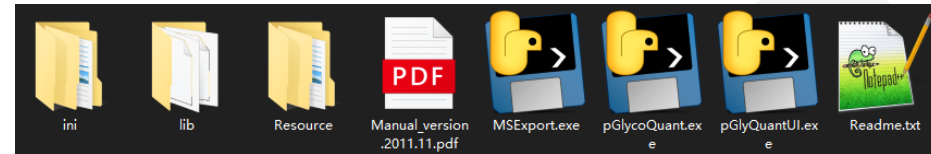
WeiQian Cao, et. al. pGlycoQuant with a deep residual network for precise and minuscule-missing-value quantitative glycoproteomics enabling the functional exploration of site-specific glycosylation. bioRxiv 2021.11.15.468561.

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

Assets

Manual_version.2021.11.pdf	359 KB
MSFileReader.3.0.Sp1.zip	37.2 MB
pGlycoQuant_V1.1.zip	308 MB
Source code (zip)	
Source code (tar.gz)	

②



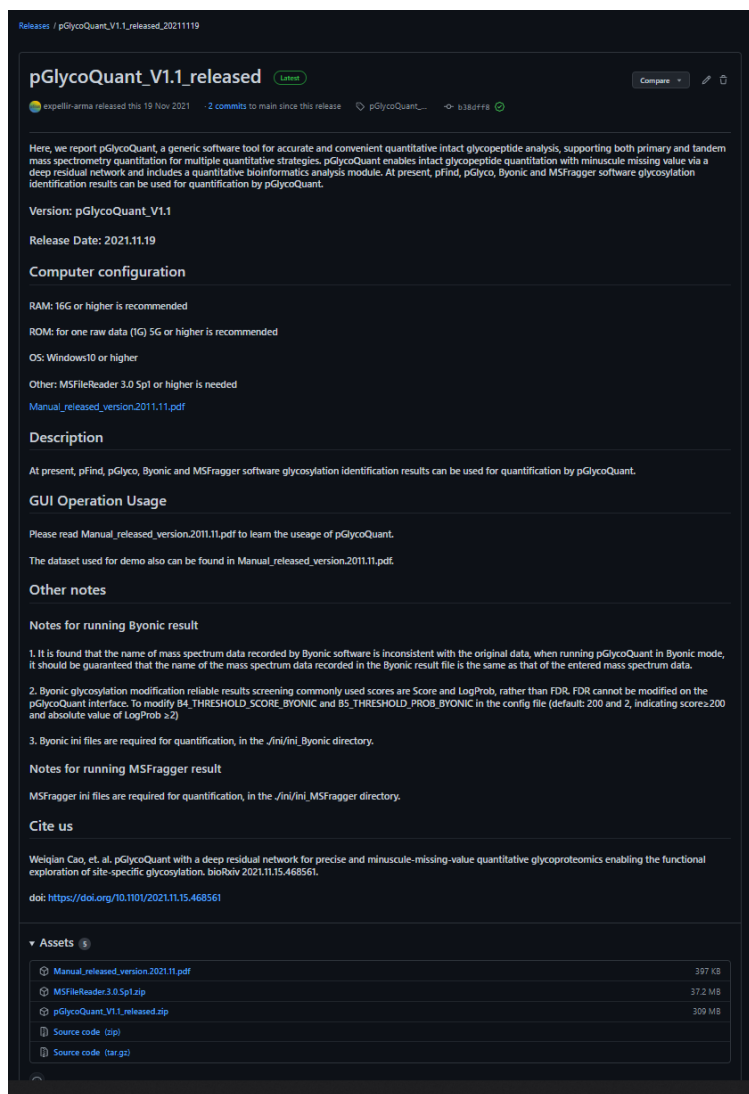
① Login <https://github.com/expelliir-arma/pGlycoQuant/releases> and download the latest version at the top of the page.

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

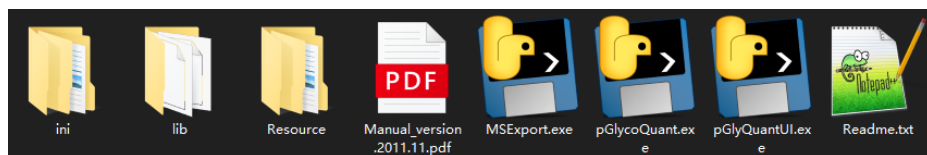
② The unzipped pGlycoQuant files.

1 pGlycoQuant download

①



②



① Login https://github.com/expellir-arma/pGlycoQuant_V1.1_released/releases and download **pGlycoQuant_V1.1_released.zip** at the bottom of this page.

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

② The unzipped pGlycoQuant files.

③ The dataset to test the software could be downloaded from MassIVE (<https://massive.ucsd.edu/>) with identifier MSV000088347. The login name of the dataset's web page (including title, description, and metadata) is MSV000088347_reviewer and password is a.

For Label Freedata, the dataset ftp://massive.ucsd.edu/raw/Raw_label-free%20HeLa-cell-Data/1902-Hela-Labelfree-01.raw and [1902-Hela-Labelfree-02.raw](ftp://massive.ucsd.edu/raw/Raw_label-free%20HeLa-cell-Data/1902-Hela-Labelfree-02.raw) could be used for testing.

③

MassIVE MSV000088347 Files

FTP Download Link for private dataset (click to copy): <ftp://MSV000088347@massive.ucsd.edu>

Note: The above URL is valid only while this dataset is still private. After publication, the permanent URL to the dataset will be:

FTP Download Link for public dataset (click to copy): <ftp://massive.ucsd.edu/MSV000088347>

Filter by Collection: [Show all](#) [ccms parameters](#) [ccms quant](#) [quant](#) [raw](#)

Dataset Files

◀ Hits 61 ~ 70 out of 70

▶ Go to

Go

Select columns

Apply Filters

File 

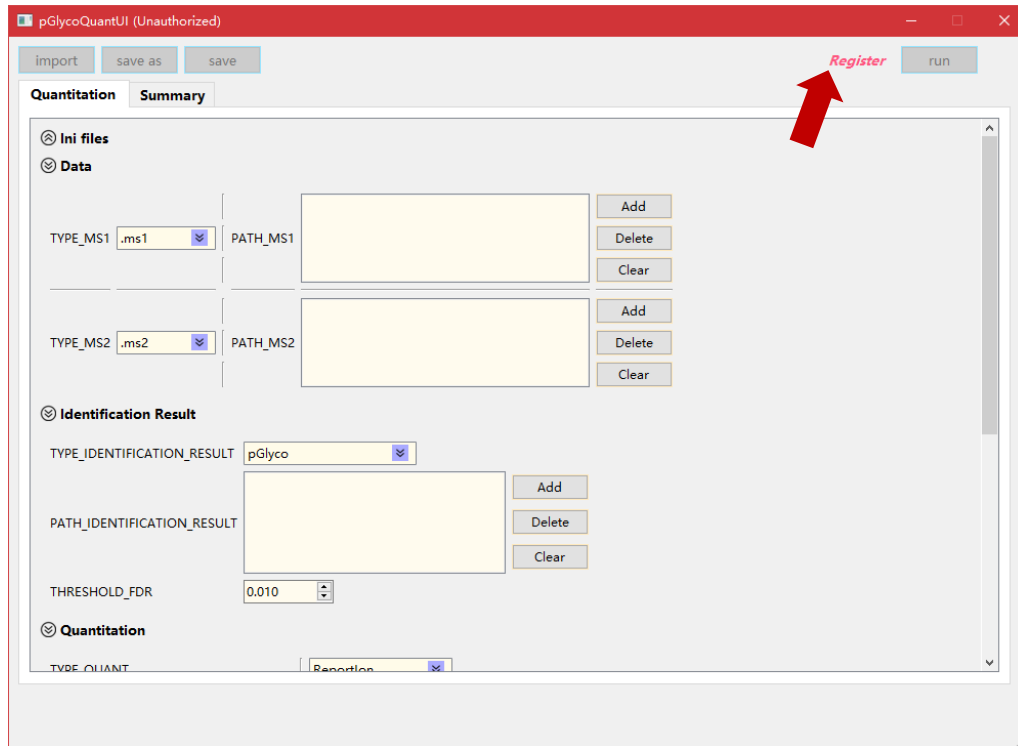
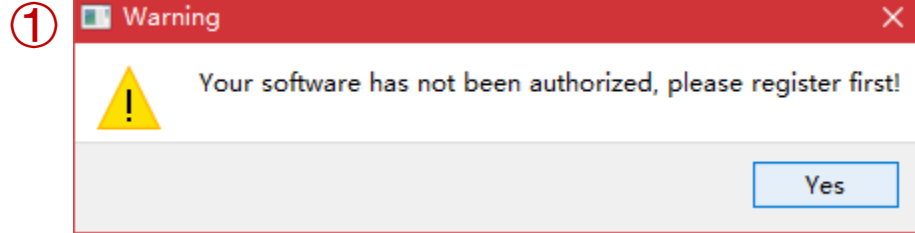
Filter By:

f.MSV000088347/raw/Raw_label-free_HeLa-cell-Data/1902-Hela-Labelfree-01.raw

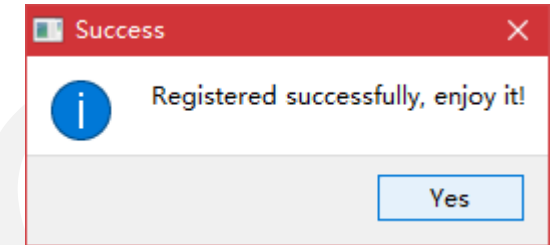
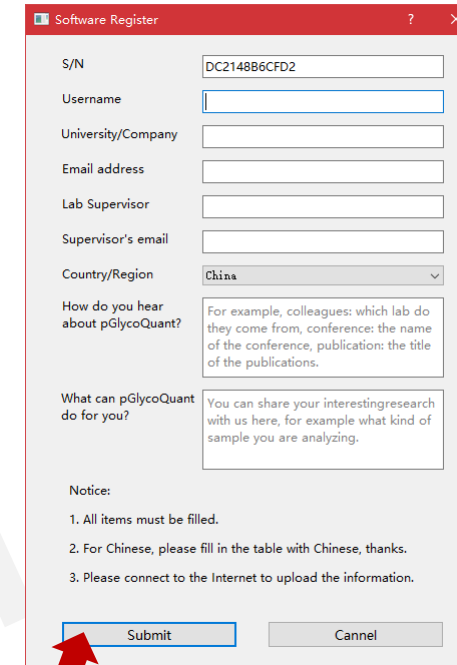
f.MSV000088347/raw/Raw_label-free_HeLa-cell-Data/1902-Hela-Labelfree-02.raw

2 Quantitation with pGlycoQuant

2.2 pGlycoQuant register



②



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

The screenshot shows the pGlyco3 software window with the 'Raw' tab selected. The interface includes a 'File Type' dropdown set to 'raw' (marked with ①), a 'Fragmentation' dropdown set to 'HCD' (marked with ②), and a 'Load Parameters' button. Below these, a table lists loaded raw files (marked with 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

To the right of the table are buttons for 'Add', 'Remove', and 'Clear All'.

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:** text box 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:** text box, 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 window 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\LabelFree\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\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\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\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\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: datapath = 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: E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlycoDB-GP-Raw1-FDR-noFiltered.txt
Process ID=25040: 12829 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 results.
- ③ The identification result file used for quantitation.

②

process2	
process1	
multiprocess_run.bat	1 KB
pGlyco.cfg	2 KB
pGlyco3.log	33 KB
pGlycoDB-GP-FDR.txt	2,784 KB
pGlycoDB-GP-FDR-Pro.txt	2,963 KB
pGlycoDB-GP-FDR-Pro-Quant.txt	3,125 KB
pGlycoDB-GP-FDR-Pro-Quant-Site.txt	3,176 KB

③

2 Quantitation with pGlycoQuant

Label Free

2.1 pGlycoQuant quantitation

①

① Ensure that the ini file paths are valid.

②

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

pQuant2.0 / pGlycoQuantUI (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

THRESHOLD_FDR 0.010

Quantitation

3 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 disable

DDALF_RT_HALF_WIN_IN_MIN 2.00

DDALF_PPM_HALF_WIN_ACCURACY_PEAK 20.00

Export

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

FLAG_CREATE_NEW_FOLDER 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**.

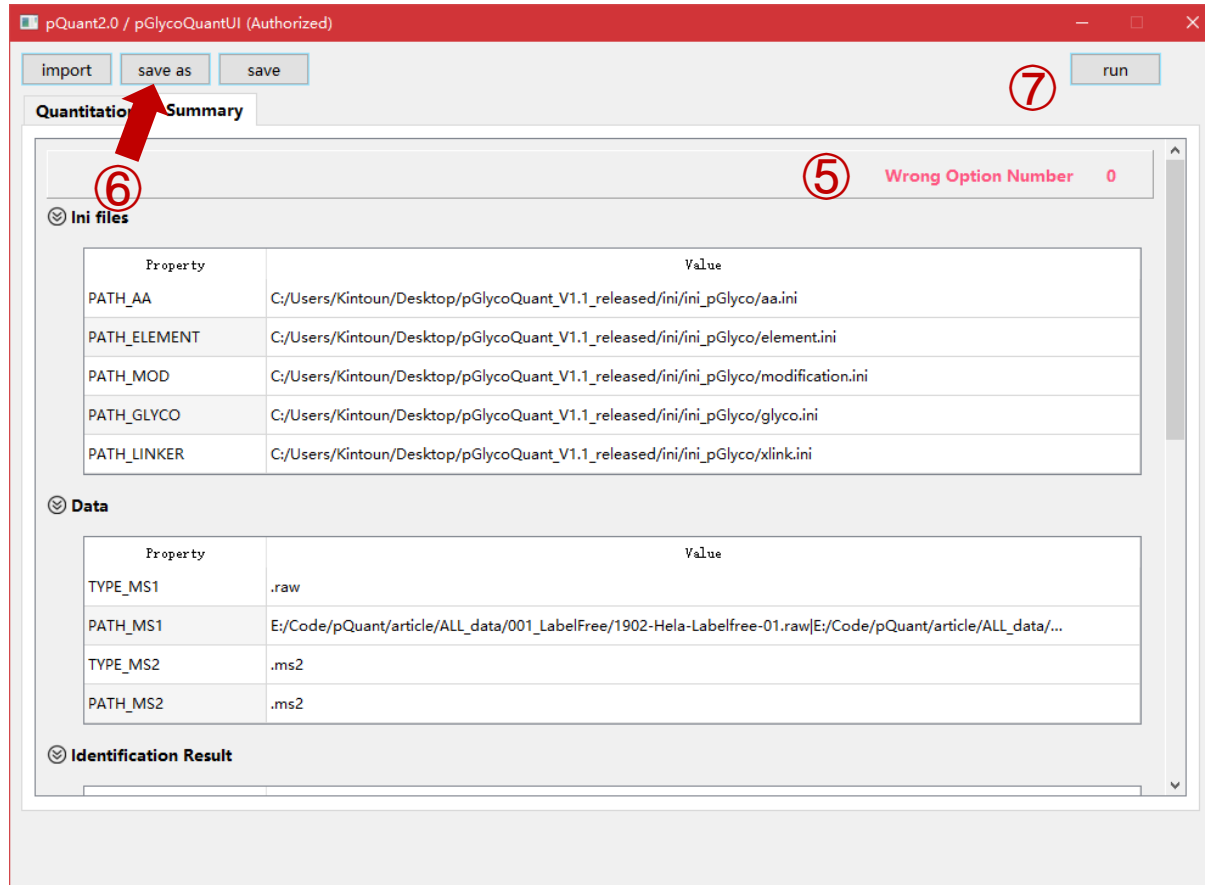
③ Set **TYPE_QUANT** as **DDA LabelFree**.

④ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

Label Free

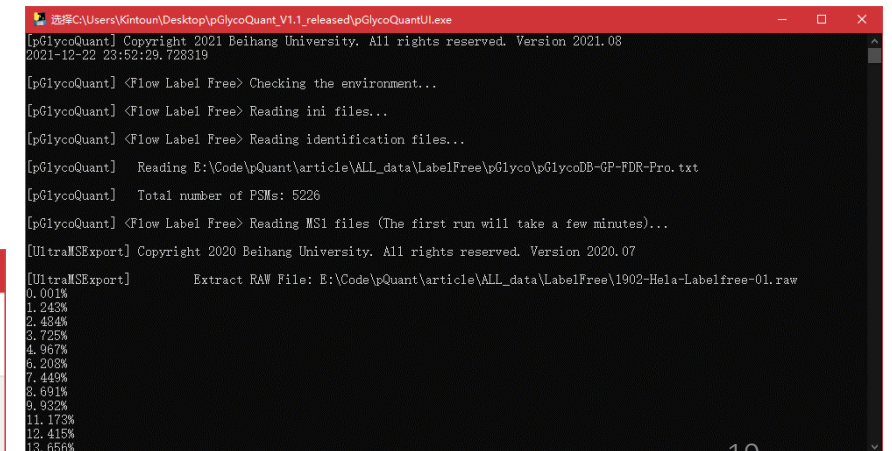
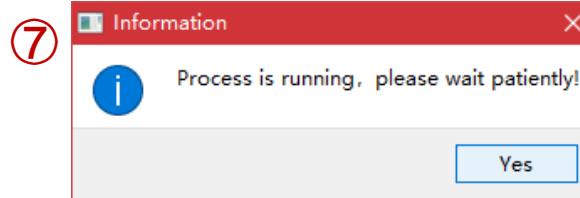
2.1 pGlycoQuant quantitation



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.

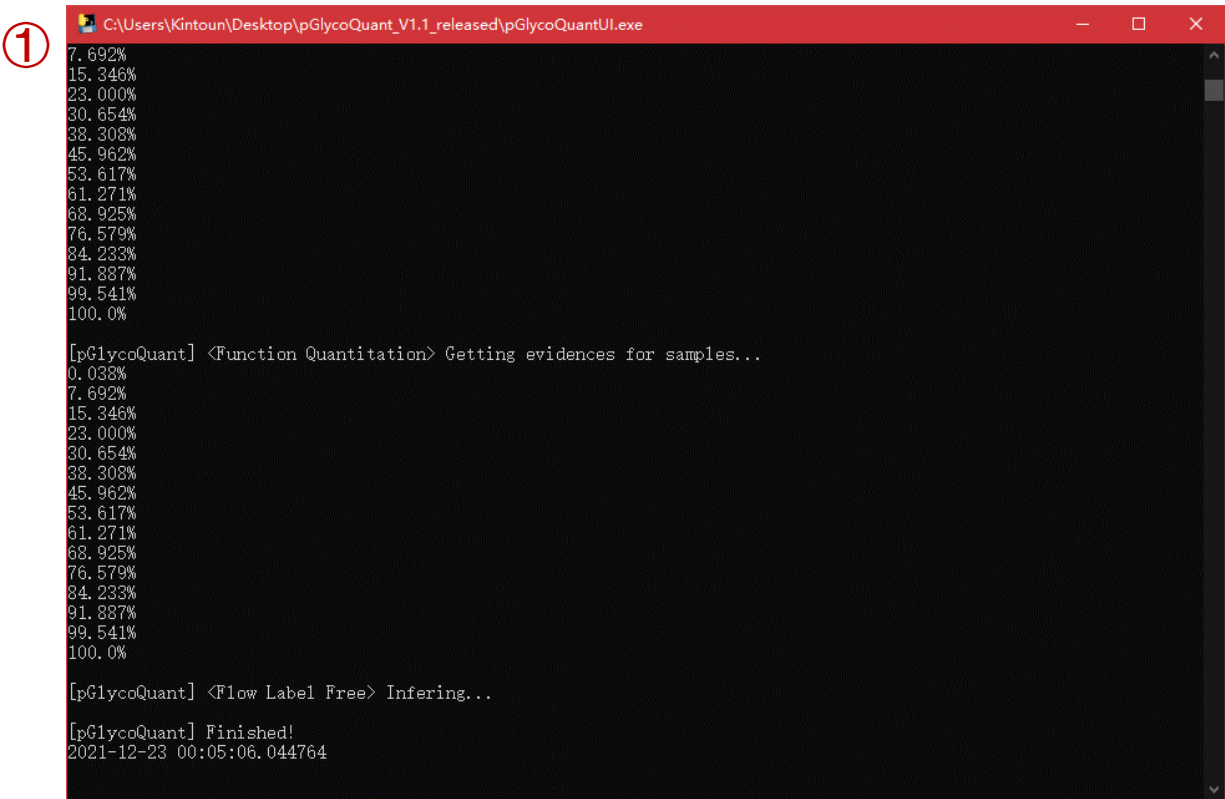
⑦ Click **run** button to start the quantitation, the progress information will be shown in the command-line interface.



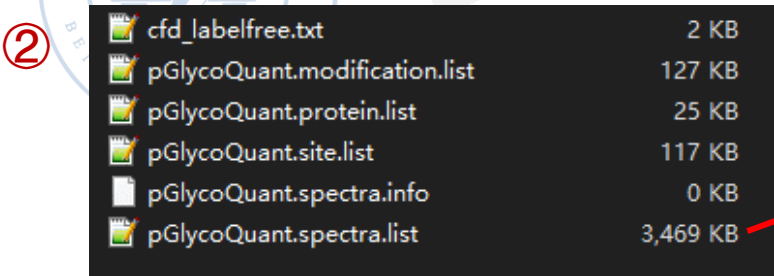
2 Quantitation with pGlycoQuant

Label Free

2.2 quantitation results



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



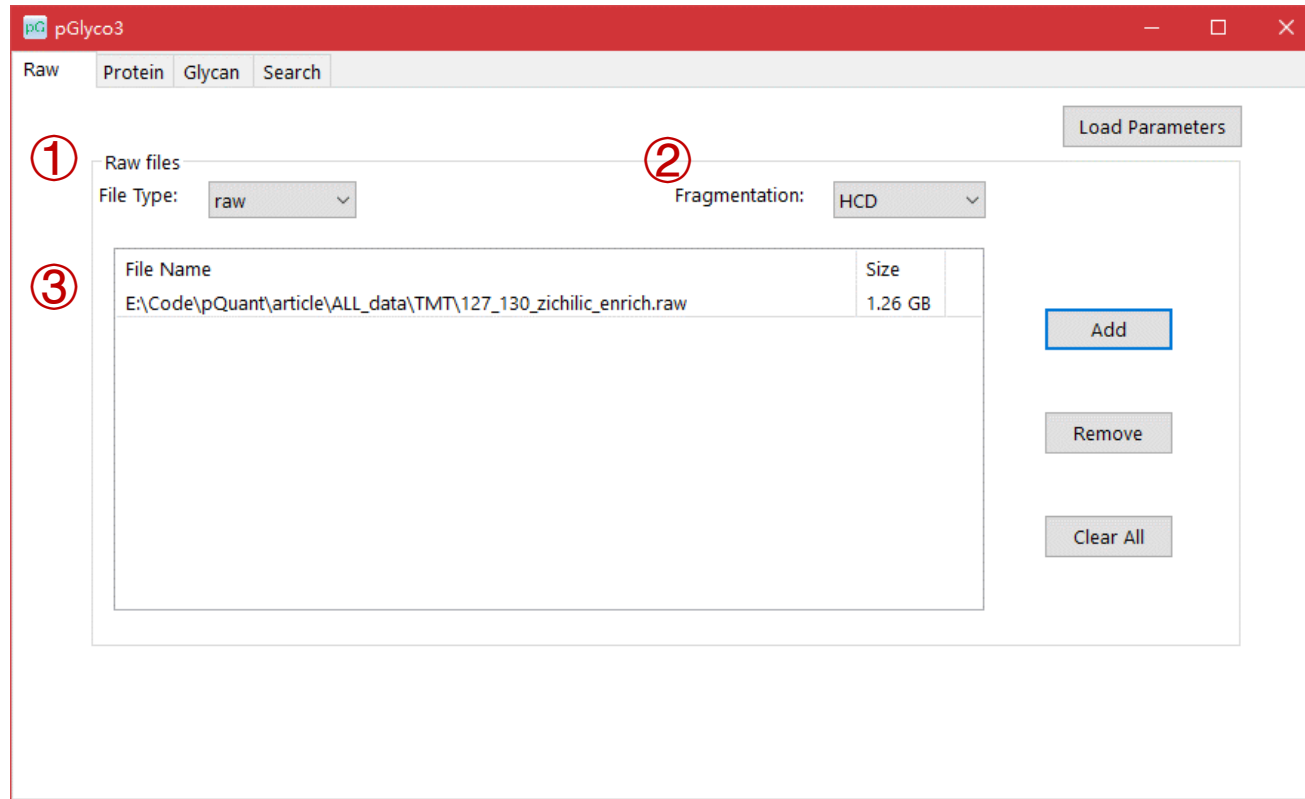
pGlycoQuant.spectra - Excel																Kite team		共享			
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pGlyco & pGlycoQuant

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** section, which contains `Carbamidomethyl[C]`, `TMT6plex[AnyN-term]`, and `TMT6plex[K]`. Below it, the **Variable Protein Modifications** section contains `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 software window with the following configuration:

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

Navigation buttons: <<, >>, Glyco: N, Modified as: (dropdown), H, pH.

- 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 pGlyco3 interface is divided into several sections. At the top, there are tabs for 'Raw', 'Protein', 'Glycan', and 'Search'. Below these, the 'Protein' tab is active, showing configuration options. On the left, 'Precursor Tolerance' is set to ± 4 ppm (labeled 1), 'Number of Processes' is set to 5 (labeled 3), and 'Glycopeptide FDR' is set to 0.01 (labeled 4). There are checkboxes for 'Percolator', 'FMM for Peptide FDR', and 'pGlycoSite: Localized Glycans Must Be in GDB'. On the right, the 'pGlycoNovo' section allows for glycan modifications: 'Glyco: H' (Max: 20), 'Glyco: N' (Max: 7), 'Glyco: F' (Max: 5), 'Glyco: A' (Max: 4), and several empty slots. The 'Allow Max Glyco Gap' is set to 3. At the bottom, the 'Output Folder' is set to 'E:\Code\pQuant\article\ALL_data\TMT\pGlyco' (labeled 5), with 'Run', 'Save', and 'Stop' buttons (labeled 6).

- 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\pGlyco3.0_build20210615\bin>pGlyco3GUI.exe
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.0_build20210615\bin>pGlyco3GUI.exe
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.0_build20210615\bin>pGlyco3GUI.exe
[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\pGlyco.cfs"
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\pGlyco3.0_build20210615\bin>pGlyco3GUI.exe
E:\Code\pQuant\article\ALL_data\pGlyco\pGlyco3.0_build20210615\bin>pGlyco3GUI.exe
[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\pGlyco.cfs"
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
\article\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.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

process1	
multiprocess_run.bat	1 KB
pGlyco.cfg	2 KB
pGlyco3.log	14 KB
pGlycoDB-GP-FDR.txt	63 KB
pGlycoDB-GP-FDR-Pro.txt	67 KB
pGlycoDB-GP-FDR-Pro-Quant.txt	71 KB
pGlycoDB-GP-FDR-Pro-Quant-Site.txt	72 KB

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

①

① Ensure that the ini file paths are valid.

②

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

pQuant2.0 / pGlycoQuantUI (Authorized)

import save as save run

Quantitation Summary

Ini files

PATH_AA pp/pGlycoQuant_V1.1_released/ini/ini_pGlyco/aa.ini Browser

PATH_ELEMENT glycoQuant_V1.1_released/ini/ini_pGlyco/element.ini Browser

PATH_GLYCO pGlycoQuant_V1.1_released/ini/ini_pGlyco/glyco.ini Browser

PATH_MOD Quant_V1.1_released/ini/ini_pGlyco/modification.ini Browser

PATH_LINKER y/pGlycoQuant_V1.1_released/ini/ini_pGlyco/xlink.ini Browser

Data

TYPE_MS1 .ms1 PATH_MS1 Add Delete Clear

TYPE_MS2 .raw PATH_MS2 p:/ALL_data/002_TMT/127_130_zichilic_enrich.raw Add Delete Clear

Identification Result

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

pQuant2.0 / pGlycoQuantUI (Authorized)

import save as save run

Quantitation Summary

Identification Result

① TYPE_IDENTIFICATION_RESULT pGlyco

② PATH_IDENTIFICATION_RESULT 002_TMT/pGlyco/pGlycoDB-GP-FDR-Pro.txt

THRESHOLD_FDR 0.010

Quantitation

③ TYPE_QUANT ReportIon

RI_PPM_HALF_WIN_ACCURACY_PEAK 1000.00

③ RI_MASS_REPORT_ION 126.127726,127.124761,128.134436,129.131471,130.141145,131.1381

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 disable

DDALF_RT_HALF_WIN_IN_MIN 2.00

DDALF_PPM_HALF_WIN_ACCURACY_PEAK 20.00

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**.

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.

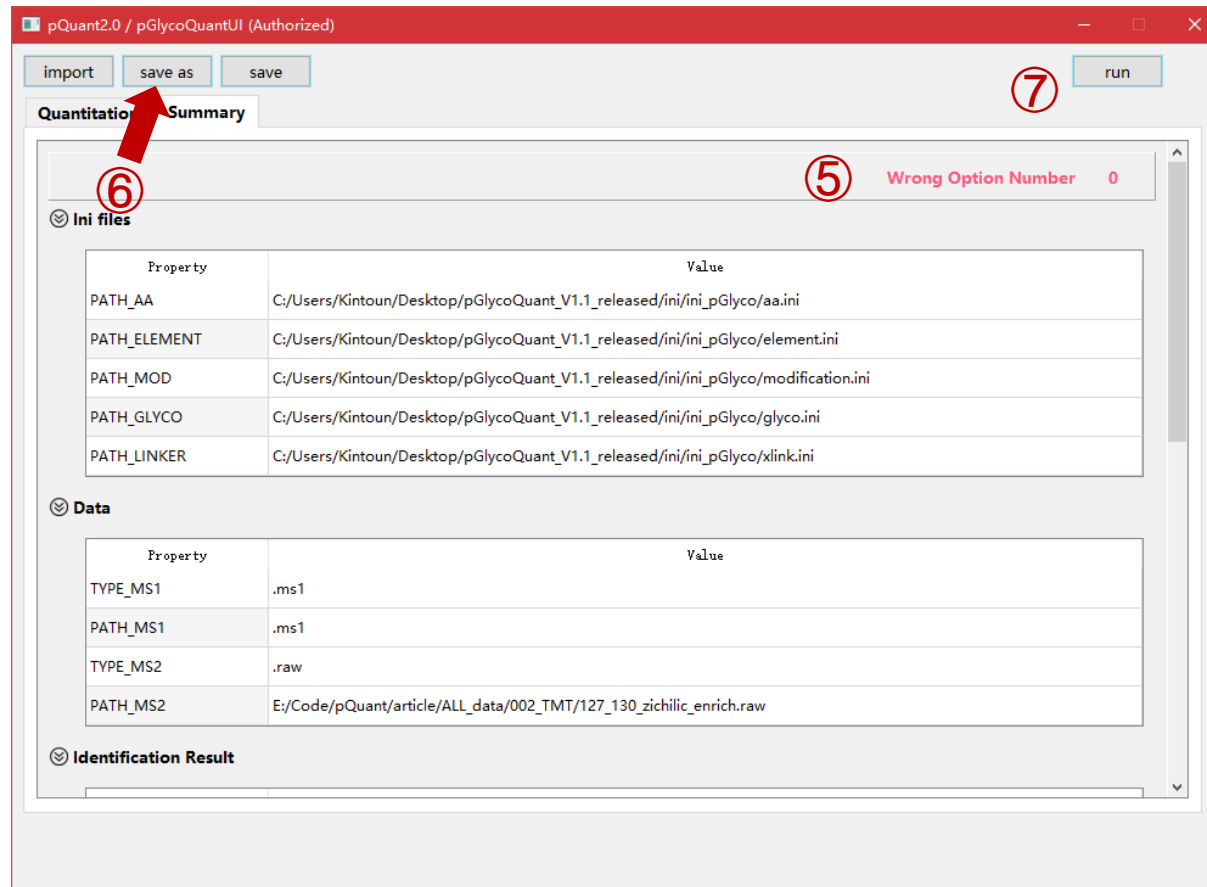
Export

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

FLAG_CREATE_NEW_FOLDER No

2 Quantitation with pGlycoQuant

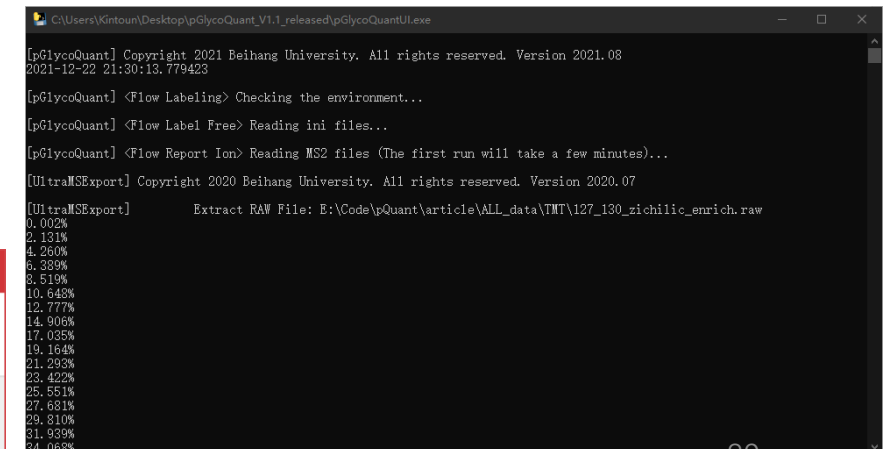
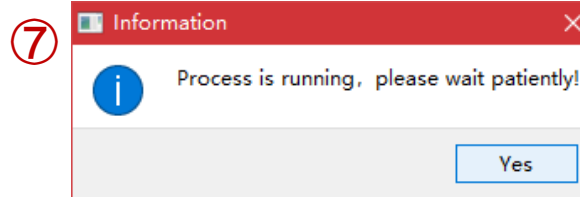
1.3 pGlycoQuant quantitation



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

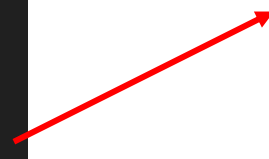
⑥ Then click **save as** button to save the config file.

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



TMT

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

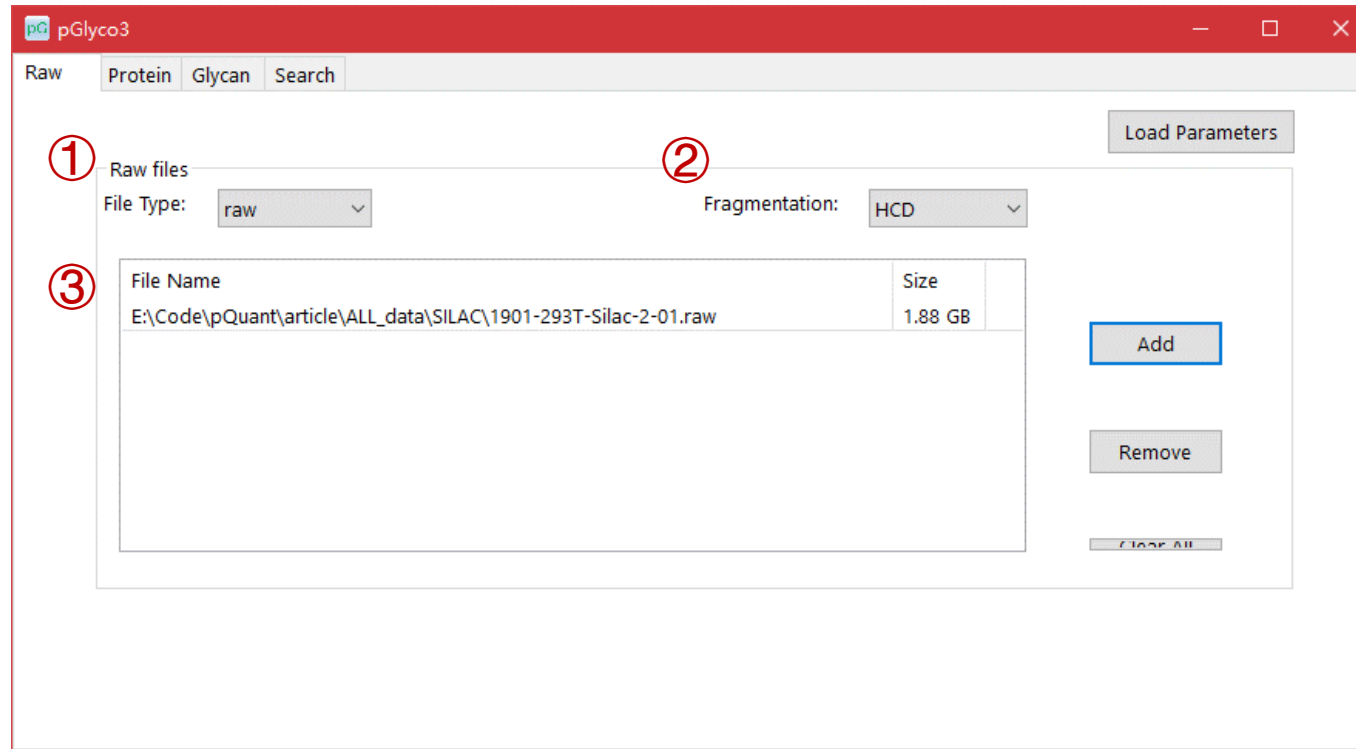
[illegible]

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:

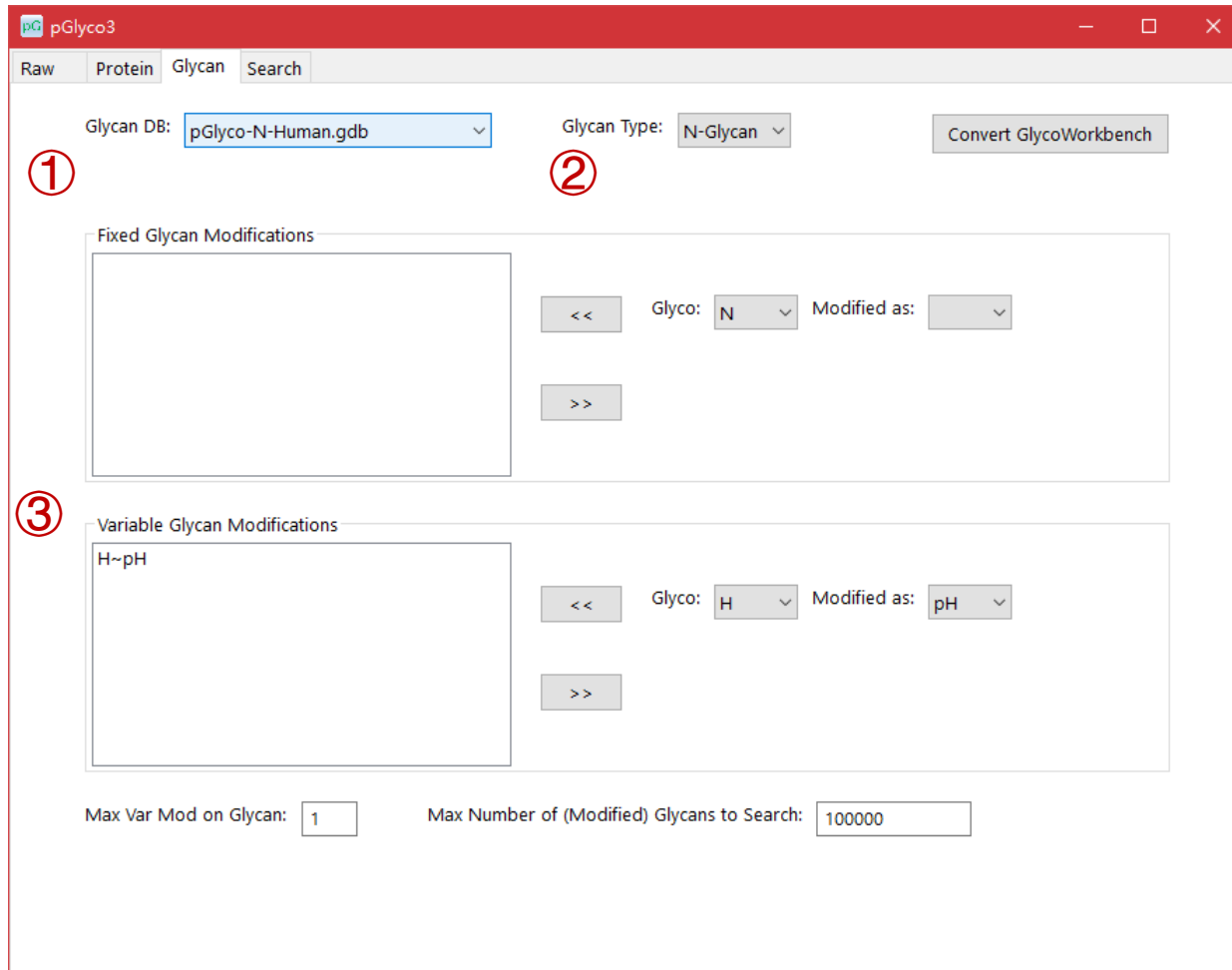
- Annotation 1:** Points to the **Fasta** field, which contains the file path `E:\Code\pQuant\article\ALL_data\SILAC\Human-H.sapiens-SP-1808_N2J_STC.fasta`.
- Annotation 2:** Points to the **Enzyme** section, where the **Name** is set to `Trypsin`, **Digestion** is set to `specific`, and **Max Miss Cleavage** is set to `2`.
- Annotation 3:** Points to the **Fixed Protein Modifications** section, which contains `Carbamidomethyl[C]`. The **Variable Protein Modifications** section below it contains `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 set to `2`, **Peptide Length** is from `6` to `40`, and **Peptide Mass** is from `600` to `4000`.

The **Modification List** on the right includes various labeled modifications such as `Label_13C(4)15N(1)[D]`, `Label_13C(5)15N(1)[E]`, `Label_13C(6)+Acetyl[K]`, and `Label_13C(6)+GlyGly[K]`.

- 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 active. 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 a large empty text box on the left and controls on the right: '<<' and '>>' buttons, a 'Glyco:' dropdown set to 'N', and a 'Modified as:' dropdown. The 'Variable' section (annotated with a red circle 3) has a text 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 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: pParse2.2 (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

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

2 Quantitation with pGlycoQuant

SILAC

1.3 pGlycoQuant quantitation

①

②

pQuant2.0 / pGlycoQuantUI (Authorized)

import save as save run

Quantitation Summary

Ini files

PATH_AA pp/pGlycoQuant_V1.1_released/ini/ini_pGlyco/aa.ini Browser

PATH_ELEMENT glycoQuant_V1.1_released/ini/ini_pGlyco/element.ini Browser

PATH_GLYCO pGlycoQuant_V1.1_released/ini/ini_pGlyco/glyco.ini Browser

PATH_MOD Quant_V1.1_released/ini/ini_pGlyco/modification.ini Browser

PATH_LINKER /pGlycoQuant_V1.1_released/ini/ini_pGlyco/xlink.ini Browser

Data

TYPE_MS1 .raw PATH_MS1 /ALL_data/003_SILAC/1901-293T-Silac-2-01.raw Add Delete Clear

TYPE_MS2 .ms2 PATH_MS2 Add Delete Clear

Identification Result

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

pQuant2.0 / pGlycoQuantUI (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

THRESHOLD_FDR 0.010

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_180 disable

DDALF_RT_HALF_WIN_IN_MIN 2.00

DDALF_PPM_HALF_WIN_ACCURACY_PEAK 20.00

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 the identification result file **pGlycoDB-GP-FDR-Pro.txt** here and set **FDR** as **0.01**.

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

The DDALL_LABEL_INFO could be

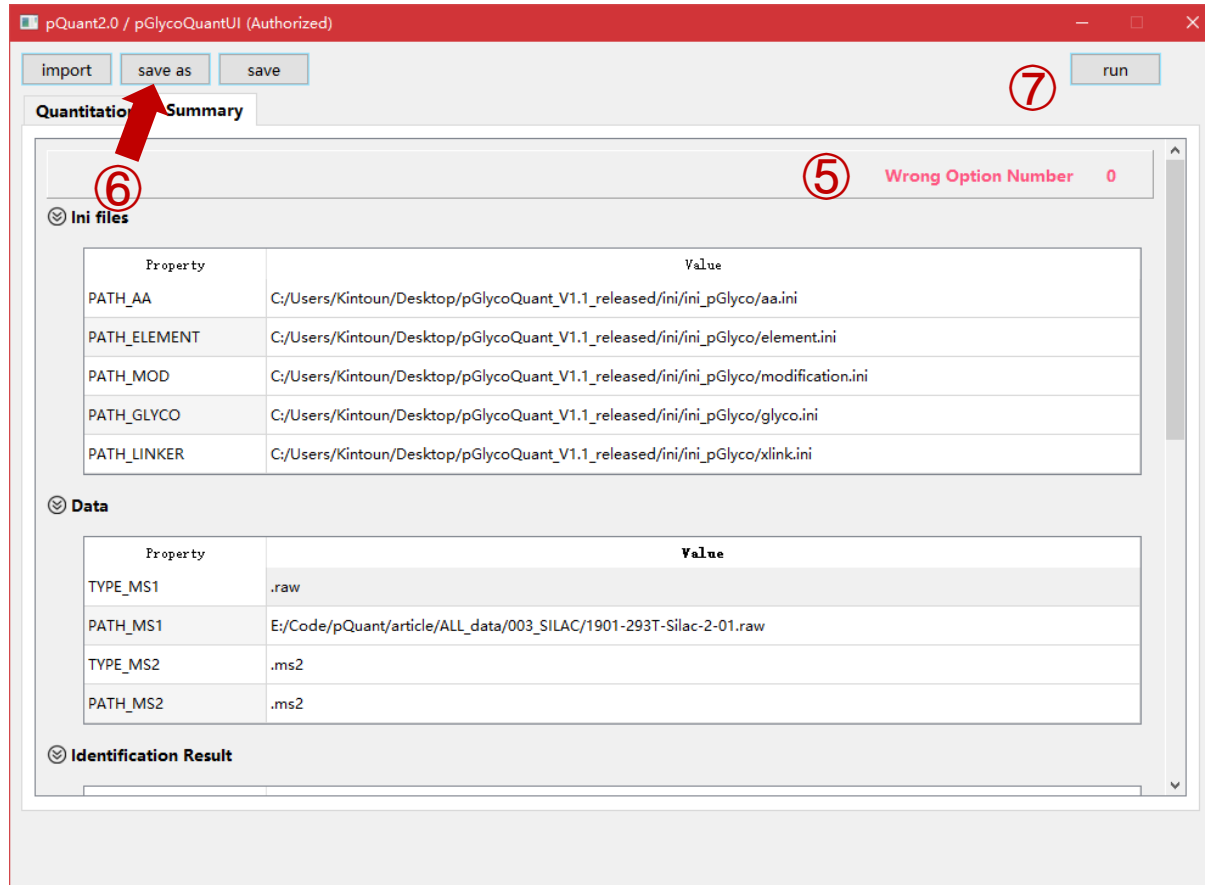
2|MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|AA:K:C:13C&AA:R:C:13C&MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|

④ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

SILAC

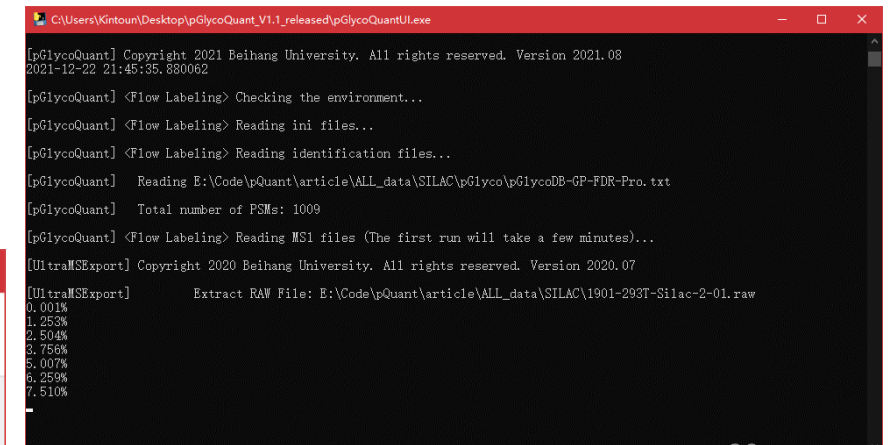
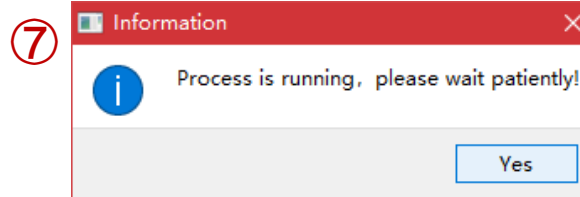
1.3 pGlycoQuant quantitation



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

SILAC

1.4 quantitation results

```
1 80.099%
81.351%
82.602%
83.854%
85.106%
86.357%
87.609%
88.860%
90.112%
91.363%
92.615%
93.866%
95.118%
96.369%
97.621%
98.872%
100.0%

[UltraMSExport] Extract Result MS1 number:7998, MS2 number:71904

[UltraMSExport] Finished!

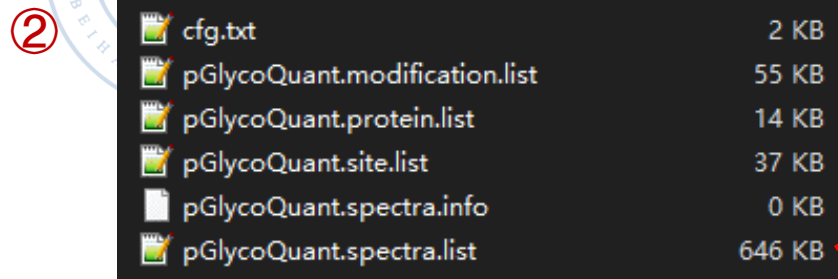
[pGlycoQuant] Creating index file for: E:\Code\pQuant\article\ALL_data\SILAC\1901-293T-Silac-2-01.ms1

[pGlycoQuant] <Flow Labeling> Quantifying...
0.10%
19.92%
39.74%
59.56%
79.39%
99.21%
100.0%

[pGlycoQuant] <Flow Labeling> Inferring...

[pGlycoQuant] Finished!
2021-12-22 22:12:34.927959
```

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



GlySpec	Peptide	Precursor	Peptide	Rank	Peptide	Mod	Peptide	Glycan	N	O	P	Q	R	S	T	U
1	1901-293T1901-293T1901-293T	30774	8313.038	2974.334	992.1162	3	1	UQTEPVAGNYYPVNT	1935.956	3.2	0.1	0.0	0.0	0.0	0.0	0.0
2	1901-293T1901-293T1901-293T	21081	5692.389	2334.024	1167.516	2	1	VQPPVITQGR	1117.6	5.2	0.0	0.0	0.0	0.0	0.0	0.0
3	1901-293T1901-293T1901-293T	6724	1820.425	2174.838	1087.923	2	1	LIDNJK	716.9307	5.2	0.0	0.0	0.0	0.0	0.0	0.0
4	1901-293T1901-293T1901-293T	25794	6969.269	3001.23	1501.119	2	1	ENVGVYLSK	1136.596	9.2	0.0	0.0	0.0	0.0	0.0	0.0
5	1901-293T1901-293T1901-293T	19506	5272.748	2209.938	1105.473	2	1	VETEMDIH10.Label.1	1155.566	4.2	0.0	0.0	0.0	0.0	0.0	0.0
6	1901-293T1901-293T1901-293T	36097	9746.257	3047.183	1016.393	3	1	DLUETHYMYR	1426.667	6.2	0.0	0.0	0.0	0.0	0.0	0.0
7	1901-293T1901-293T1901-293T	43785	11814.49	3032.299	1011.438	3	1	GVFTTETGQPLIG	1573.858	5.2	0.0	0.0	0.0	0.0	0.0	0.0
8	1901-293T1901-293T1901-293T	18015	4870.707	3228.348	1076.787	3	1	VHTGQVIYR	1363.712	9.2	0.0	0.0	0.0	0.0	0.0	0.0
9	1901-293T1901-293T1901-293T	23983	6479.312	2861.203	954.4059	3	1	TCDWLPK5.2.Carbam	1822.832	3.2	0.1	0.0	0.0	0.0	0.0	0.0
10	1901-293T1901-293T1901-293T	29213	7889.791	2322.023	1161.515	2	1	VAGFLTV10.Label.1	1105.598	5.2	0.0	0.0	0.0	0.0	0.0	0.0
11	1901-293T1901-293T1901-293T	23979	6478.165	3392.463	1131.492	3	1	LEITGTGTHAQK	1689.881	9.2	0.0	0.0	0.0	0.0	0.0	0.0
12	1901-293T1901-293T1901-293T	15949	4313.971	2792.133	931.3824	3	1	VQSPLSK8.Carbam	1226.571	5.3	0.1	0.0	0.0	0.0	0.0	0.0
13	1901-293T1901-293T1901-293T	5608	1519.05	2533.024	1267.015	2	1	LIDNITEK	1074.579	5.2	0.0	0.0	0.0	0.0	0.0	0.0
14	1901-293T1901-293T1901-293T	23481	6342.825	4526.897	1509.637	3	1	VASVINI18.Carbam	2027.993	7.6	0.1	0.0	0.0	0.0	0.0	0.0
15	1901-293T1901-293T1901-293T	14606	3948.165	2412.111	804.7083	3	1	LKPLFK	1171.5803	7.2	0.0	0.0	0.0	0.0	0.0	0.0
16	1901-293T1901-293T1901-293T	2724	737.2411	2390.956	1195.982	2	1	AAAGSLR	688.3737	8.2	0.0	0.0	0.0	0.0	0.0	0.0
17	1901-293T1901-293T1901-293T	22586	6101.251	2643.014	1322.011	2	1	IFSSCSAE5.Carbam	1426.589	5.2	0.0	0.0	0.0	0.0	0.0	0.0
18	1901-293T1901-293T1901-293T	40297	10876.03	3300.459	1100.824	3	1	VSTFTVQAI17.Carbam	2262.082	3.2	0.1	0.0	0.0	0.0	0.0	0.0
19	1901-293T1901-293T1901-293T	11796	3188.659	2251.874	1131.44	2	1	PTMKER	883.3978	6.2	0.0	0.0	0.0	0.0	0.0	0.0
20	1901-293T1901-293T1901-293T	29222	7919.221	2919.209	1460.108	2	1	GLTLTFSF11.Label.1	1216.628	8.2	0.0	0.0	0.0	0.0	0.0	0.0
21	1901-293T1901-293T1901-293T	14423	3988.73	2562.124	1281.565	2	1	LKPLFK	859.54	8.2	0.0	0.0	0.0	0.0	0.0	0.0
22	1901-293T1901-293T1901-293T	38243	10324.07	3564.587	1188.867	3	1	HLASNPTEPATITFAA	2024.056	7.2	0.0	0.0	0.0	0.0	0.0	0.0
23	1901-293T1901-293T1901-293T	24274	6558.746	3236.433	1079.483	3	1	LEITGTGTY15.Label.1	1695.901	7.2	0.0	0.0	0.0	0.0	0.0	0.0
24	1901-293T1901-293T1901-293T	28054	7564.818	2332.039	1166.523	2	1	GLSLVLYV10.Label.1	1115.615	5.2	0.0	0.0	0.0	0.0	0.0	0.0
25	1901-293T1901-293T1901-293T	5302	1436.747	2124.995	709.0033	3	1	LIDNITEK6.Label.13	1086.619	3.2	0.1	0.0	0.0	0.0	0.0	0.0
26	1901-293T1901-293T1901-293T	7391	2000.378	2329.997	1165.502	2	1	IATLAQA9.Label.13	951.5201	6.2	0.0	0.0	0.0	0.0	0.0	0.0
27	1901-293T1901-293T1901-293T	22530	6885.956	3807.609	952.6578	4	1	FTFTSHTPK14.Carbam	2429.13	6.2	0.0	0.0	0.0	0.0	0.0	0.0
28	1901-293T1901-293T1901-293T	7261	1964.902	2338.015	780.0099	3	1	DITHSDK11.Label.1	1299.638	3.2	0.1	0.0	0.0	0.0	0.0	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

pGlyco

pFind

Byonic

PD

pGlycoOLD

pLink

MSFragger

MSFragger glyco-N

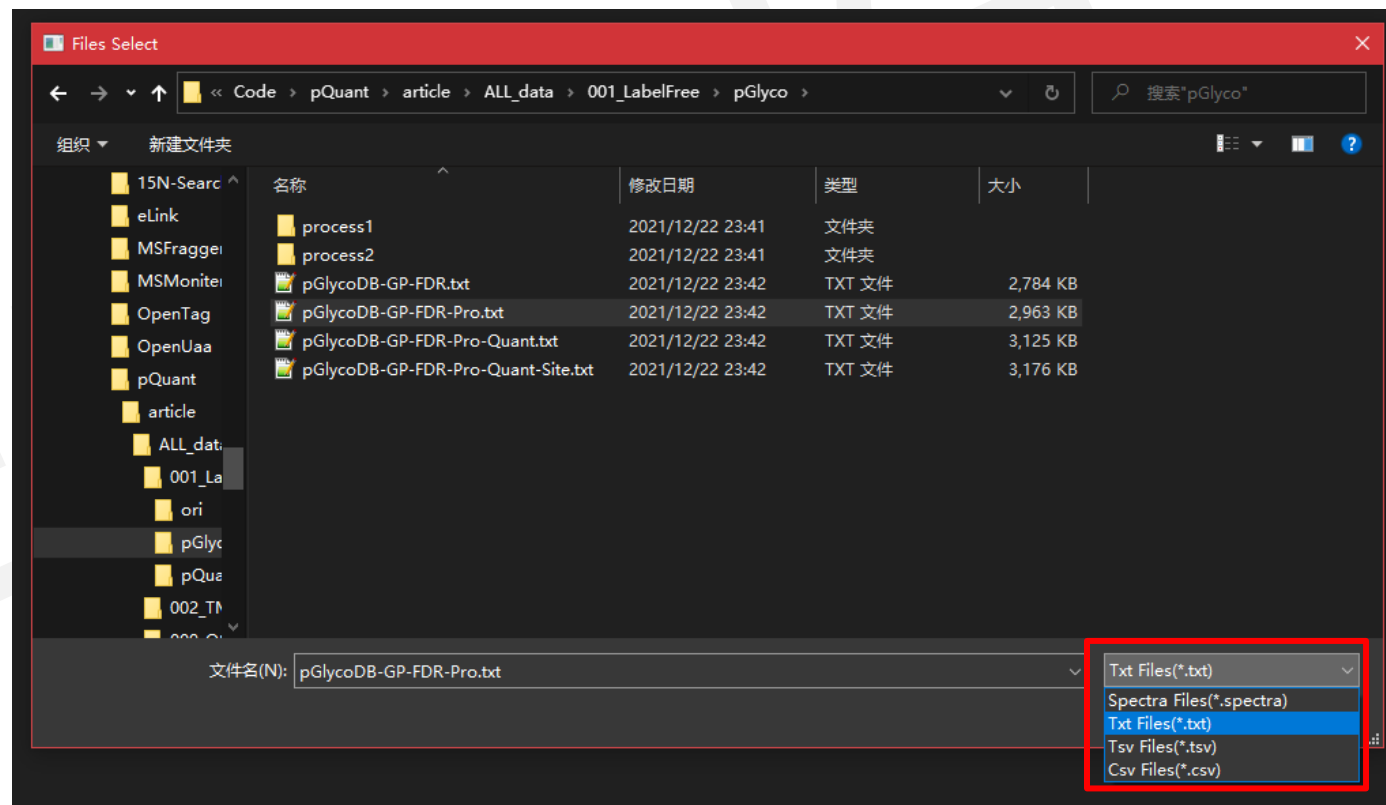
Add

Delete

Clear

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













MSFragger glyco-N

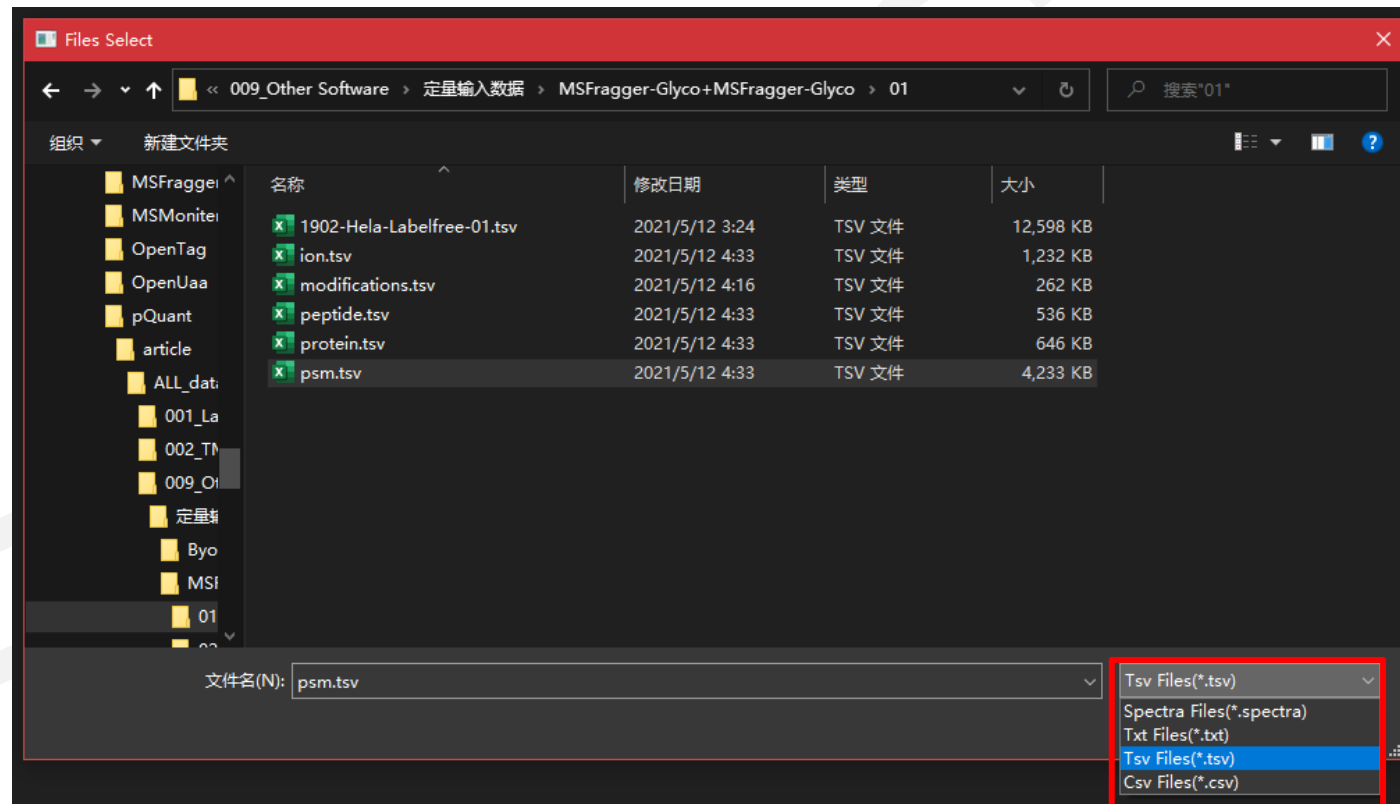
Add

Delete

Clear

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

PATH_IDENTIFICATION_RESULT

THRESHOLD_FDR

Add

Delete

Clear

Byonic

pFind

pGlyco

PD

pGlycoOLD

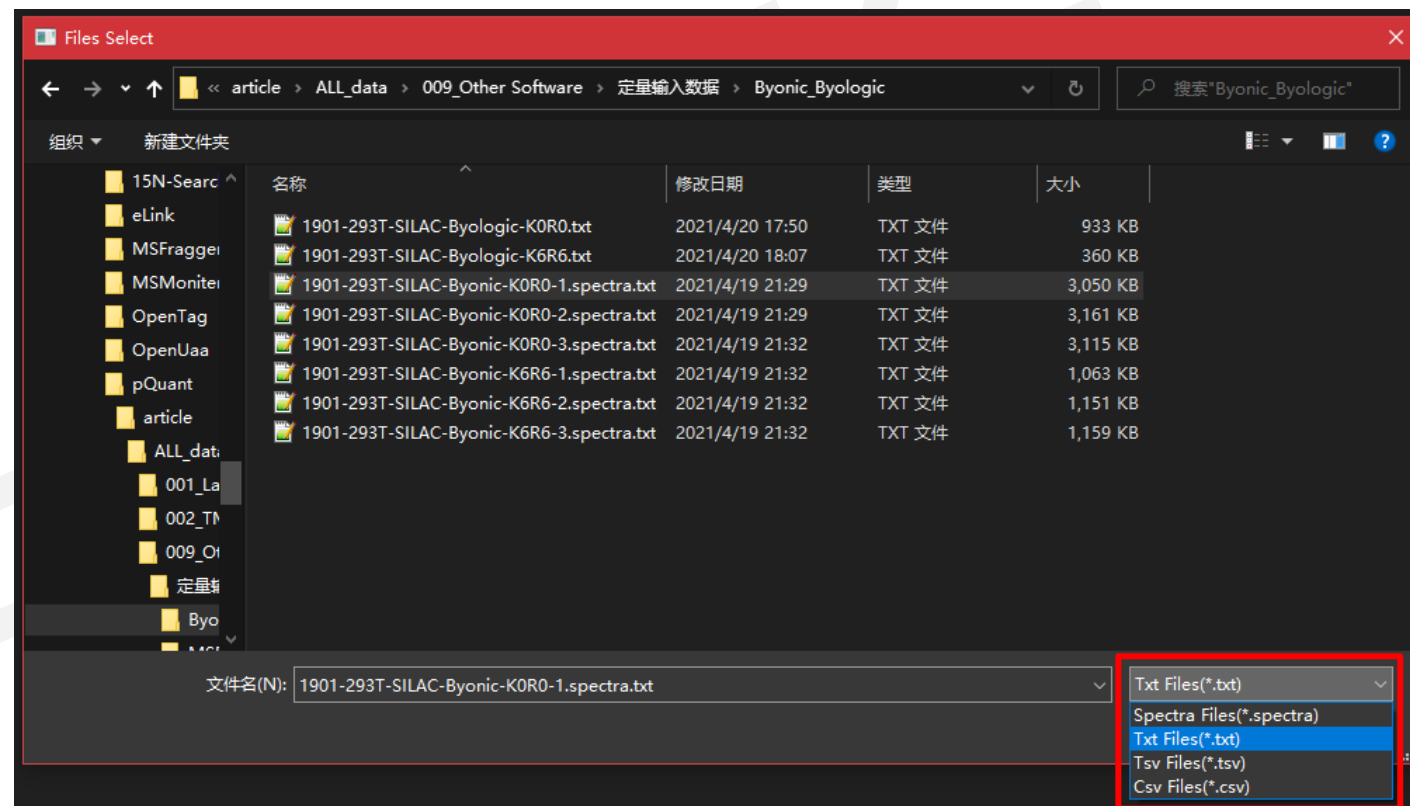
pLink

MSFragger

MSFragger glyco-N

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



Thanks!

