

pGlycoQuant Manual

version.2021.11

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 $\text{score} \geq 200$ and absolute value of $\text{LogProb} \geq 2$).
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.

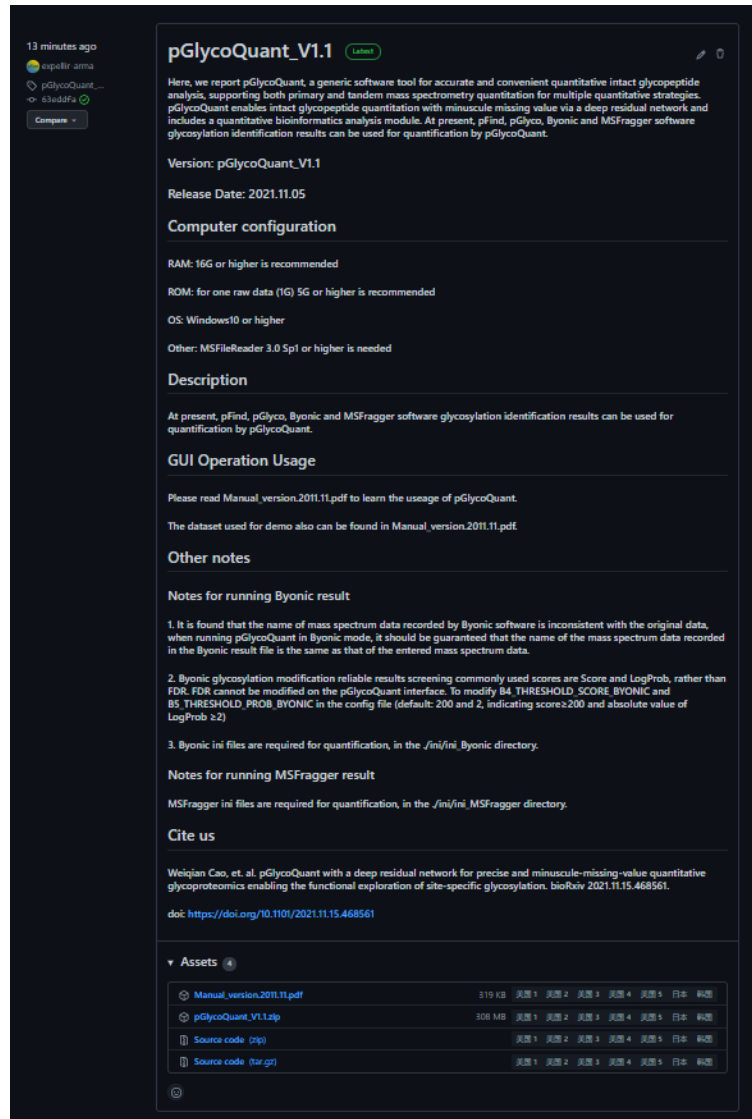
doi: <https://doi.org/10.1101/2021.11.15.468561>

A quantitation example for DDA Label

Free data after pGlyco identification

1 pGlycoQuant download

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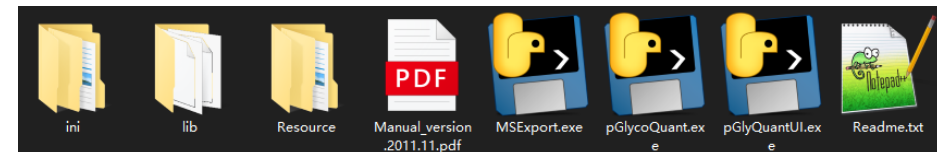
① Login <https://github.com/expellir-arma/pGlycoQuant/releases> and download

pGlycoQuant_V1.1.zip at the bottom of this page.

② The unzipped pGlycoQuant files.

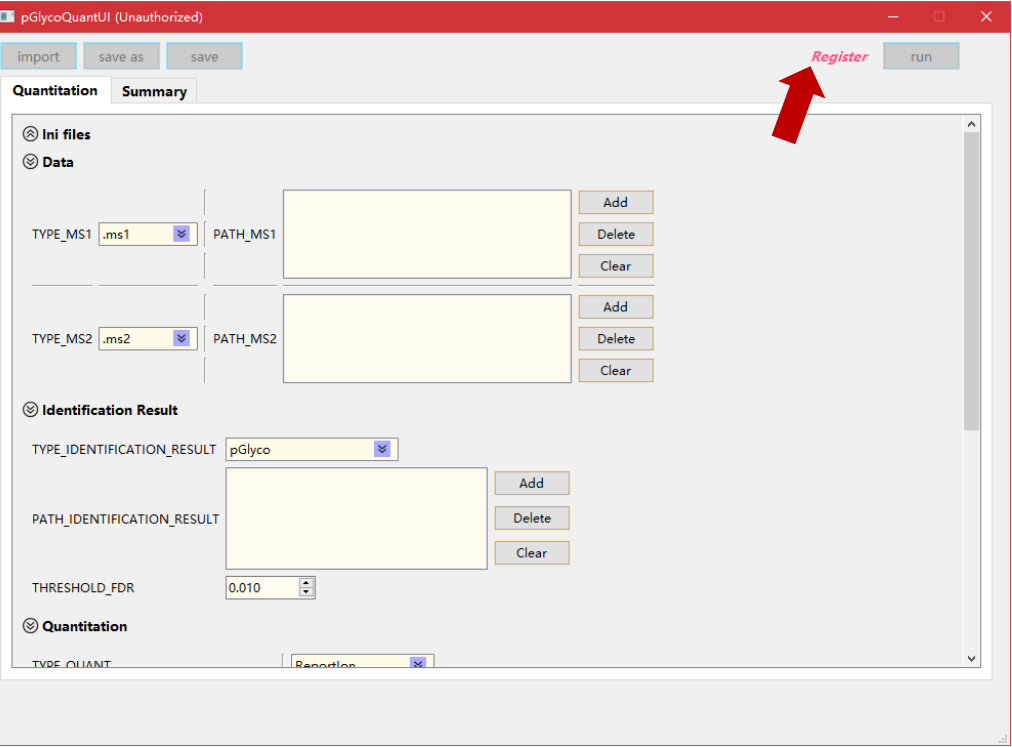
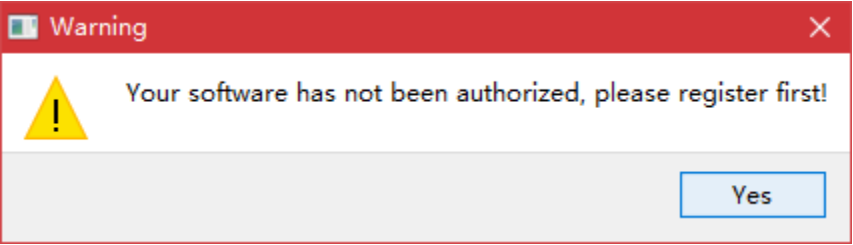
The dataset to test the software could be Label Free raw data.

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2 pGlycoQuant register

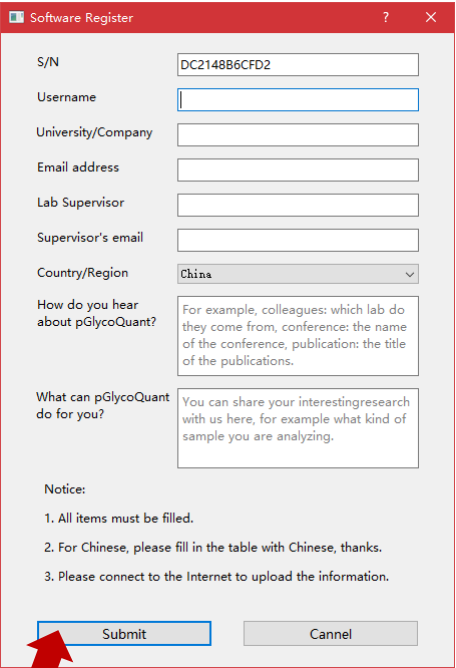
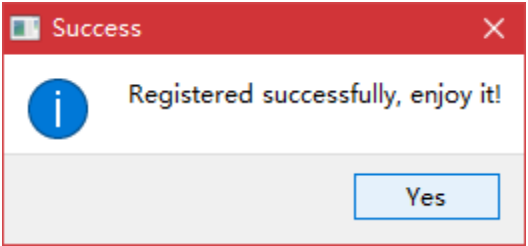
①



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.

②

A "Software Register" dialog box with a red title bar. It contains several input fields: "S/N" (with value "DC2148B6CFD2"), "Username", "University/Company", "Email address", "Lab Supervisor", "Supervisor's email", and "Country/Region" (with value "China"). There are two text areas: "How do you hear about pGlycoQuant?" and "What can pGlycoQuant do for you?". At the bottom, there are "Submit" and "Cancel" buttons. A red arrow points to the "Submit" button.

3 pGlycoQuant quantitation

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pGlycoQuantUI (Authorized)

import save as save run

Quantitation Summary

Ini files

PATH_AA normal\OGP\pGlycoQuant_V1.1\ini\ini_pGlyco\aa.ini Browser

PATH_ELEMENT normal\OGP\pGlycoQuant_V1.1\ini\ini_pGlyco\element.ini Browser

PATH_GLYCO normal\OGP\pGlycoQuant_V1.1\ini\ini_pGlyco\glyco.ini Browser

PATH_MOD normal\OGP\pGlycoQuant_V1.1\ini\ini_pGlyco\modification.ini Browser

PATH_LINKER normal\OGP\pGlycoQuant_V1.1\ini\ini_pGlyco\mlink.ini Browser

②

Data

TYPE_MS1 .raw PATH_MS1 E:/Code/pQuant/article/dataset/RAW/1902-Hel... Add
E:/Code/pQuant/article/dataset/RAW/1902-Hel... 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.

3 pGlycoQuant quantitation

The screenshot shows the pGlycoQuantUI (Authorized) window. At the top are buttons for 'import', 'save as', 'save', and 'run'. Below these are two tabs: 'Quantitation' and 'Summary'. The 'Quantitation' tab is active and contains the following fields:

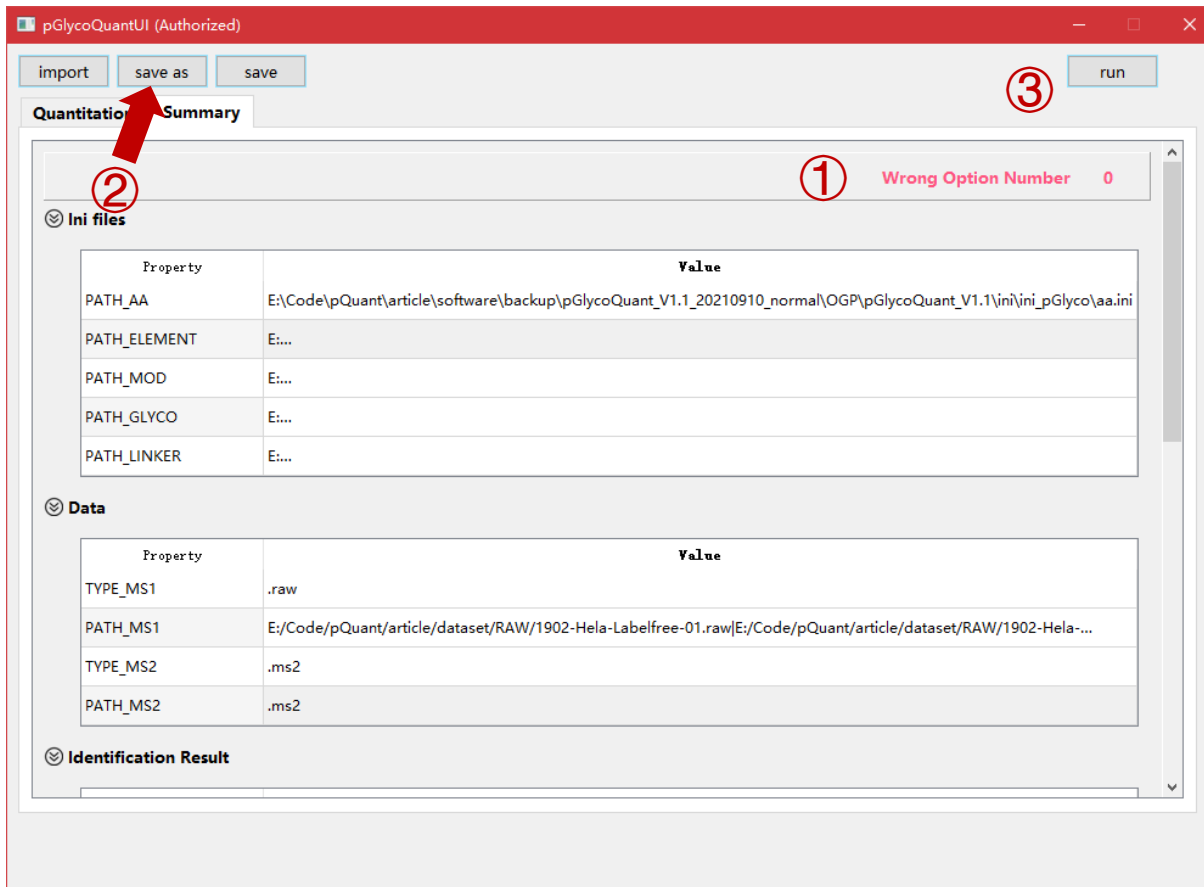
- Identification Result**
 - TYPE_IDENTIFICATION_RESULT**: A dropdown menu set to 'pGlyco' (annotated with ①).
 - PATH_IDENTIFICATION_RESULT**: A text box containing 'E:/Code/pQuant/article/dataset/iden_res/p' (annotated with ②). To its right are 'Add', 'Delete', and 'Clear' buttons.
 - THRESHOLD_FDR**: A numeric input field set to '0.010'.
- Quantitation**
 - TYPE_QUANT**: A dropdown menu set to 'DDA LabelFree' (annotated with ③).
 - RI_PPM_HALF_WIN_ACCURACY_PEAK**: A numeric input field set to '1000.00'.
 - RI_MASS_REPORT_ION**: A text box containing '127.11, 130.11'.
 - DDALL_RT_HALF_WIN_IN_MIN**: A numeric input field set to '2.00'.
 - DDALL_PPM_HALF_WIN_ACCURACY_PEAK**: A numeric input field set to '20.00'.
 - DDALL_LABEL_INFO**: A text box containing '2[NONE]AA:R:N:15N&AA:R:C:13C&AA:K:C:13C&AA:K:N:15N'.
 - DDALL_FLAG_CALIBRATION_18O**: A dropdown menu set to 'disable'.
 - DDALF_RT_HALF_WIN_IN_MIN**: A numeric input field set to '2.00'.
 - DDALF_PPM_HALF_WIN_ACCURACY_PEAK**: A numeric input field set to '20.00'.

At the bottom of the window is the 'Export' section:

- PATH_EXPORT**: A text box containing 'C:\pGlycoQuant\' (annotated with ④). To its right is a 'Browser' button.
- FLAG_CREATE_NEW_FOLDER**: A dropdown menu set to '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** (the identification result by using pGlyco) here and set **FDR** as **0.01**.
- ③ Set **TYPE_QUANT** as **DDA LabelFree**.
- ④ Set the Output Folder for saving the quantitation results.

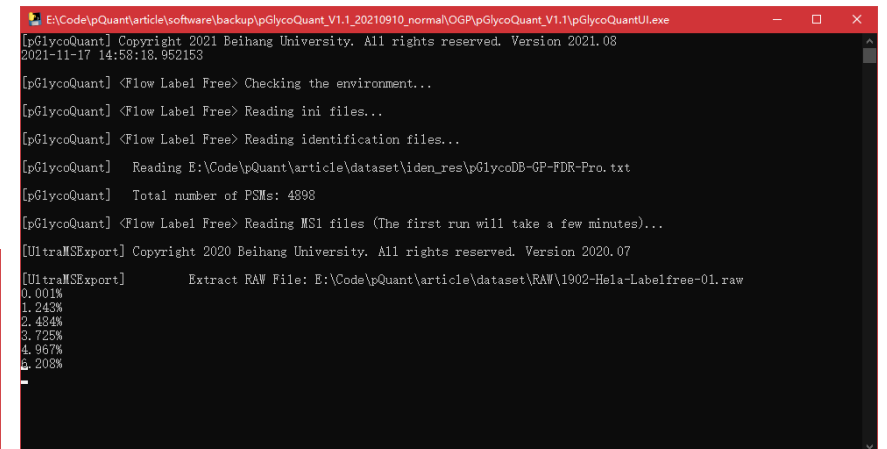
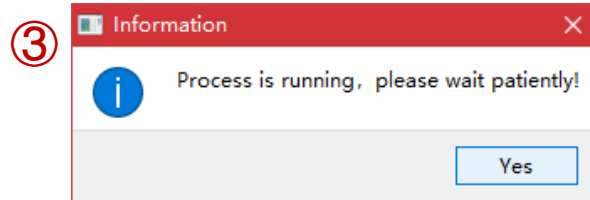
3 pGlycoQuant quantitation



① Click **Summary** button and make sure that the **Wrong Option Number** is 0.

② Then click **save as** button to save the config file.

③ Click **run** button to start the quantitation, the progress information will be shown in the command-line interface.



4 quantitation results

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```
E:\Code\pQuant\article\software\backup\pGlycoQuant_V1.1_20210910_normal\OGP...
49.040%
57.207%
65.374%
73.540%
81.707%
89.873%
98.040%
100.0%

[pGlycoQuant] <Function Quantitation> Getting evidences for samples...
0.041%
8.207%
16.374%
24.541%
32.707%
40.874%
49.040%
57.207%
65.374%
73.540%
81.707%
89.873%
98.040%
100.0%






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

[pGlycoQuant] Finished!
2021-11-17 15:10:05.724864
```

① The completed information.

② The quantitation results.

②

 pGlycoQuant.modification.list	116 KB
 pGlycoQuant.protein.list	24 KB
 pGlycoQuant.site.list	102 KB
 pGlycoQuant.spectra.info	0 KB
 pGlycoQuant.spectra.list	3,090 KB

Thanks!