

pGlycoQuant Manual

version.202211



Version: pGlycoQuant_v202211

Release Date: 2022.11.07

Computer configuration

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

RAM: 16G or higher is recommended

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

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

Other: 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 add THRESHOLD_SCORE_BYONIC=XXX and THRESHOLD_PROB_BYONIC=XXX 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 Register



1 Identification with pGlyco

1.1 pGlyco download

①

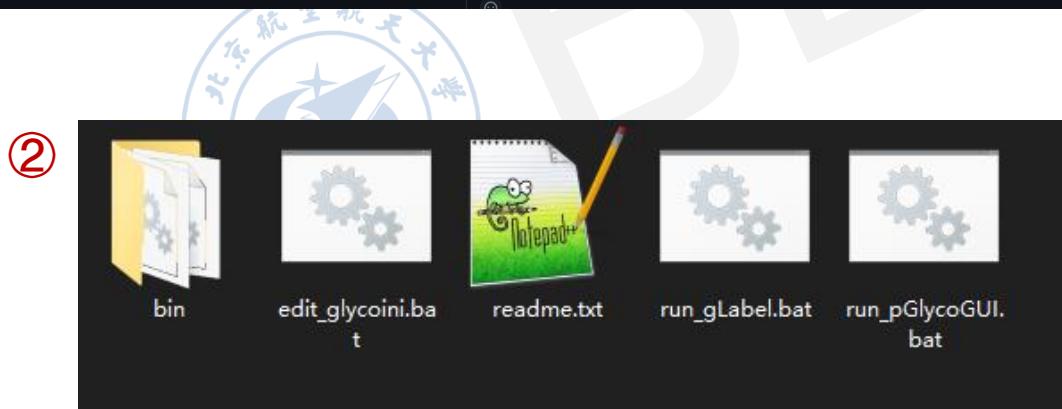
The screenshot shows the GitHub release page for 'pGlyco3.0_build20210615'. At the top, there's a header with 'Code', 'Issues 10', 'Pull requests', 'Actions', 'Projects 1', 'Wiki', 'Security', and 'Insights'. Below that is a 'Releases' tab and a 'Tags' tab. A search bar says 'Find a release'. The main content area shows the release details: 'pGlyco3.0_build20210615' (Latest), dated 16 Jun 2021, by jalew188. It includes a list of 7 items under 'What's new': 1. [FEATURE] 2+ c/z ions for 3+ or higher precursors are considered for SSGL (site-specific glycan localization) in pGlycoSite. 2. [FIX] Multi-site GDB bug fixed. This bug generates wired glycans in Multi-Site-O-Glycan.gdb before pGlyco3.0_r3_build20210124. 3. [FEATURE] Generating joint Y ions for multiple glycans (trees) using the DFS algorithm (after pGlyco3.0_r4_build20210327). 4. [FIX] "Check GDB" when SSGL sometimes does not check GDB (it depends on some conditions). Now we perform this before SSGL. 5. [FIX] Glycan encoding bug in pGlycoSite is fixed. 6. [FIX] Other minor bugs are fixed in identification and SSGL. 7. [FEATURE] Under Testing: 2*Fuc will be corrected as NeuAc if NeuAc-glycopeptide precursor signal is found in the MS1 spectrum and 274/292 ions were found in MS2. A column named "CorrectedGlycan(x,x,x)" is added in the result file. We are working on the better method for this correction. Below the release notes is a section titled 'Assets' with three items: 'pGlyco3.0_build20210615.zip' (92.6 MB), 'Source code (zip)', and 'Source code (tar.gz)'.

① Login

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

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

② The unzipped pGlyco files.



1 Identification with pGlyco

1.2 pGlyco register

pGlyco3

Sorry, your license is invalid.

確定

pGlyco3 License Dialog

*Activation code: 4A5BEF5141901C9801465A373F0FCEE9

*User name: First name Last name

*University / Company:

*Work email address:

*Lab leader / Supervisor:

*Supervisor's email:

*Country / Region: China (Mainland)

*How do you hear about pGlyco?

For example, colleagues: which lab do they come from, conference: the name of the conference, publication: the title of the publication.

*What can pGlyco3 do for you?

You can share your interesting research with us here, for example what kind of glycopeptides you are analyzing.

Notice:

1. All items must be filled.
2. For Chinese, please fill in the table with Chinese, thanks.
3. Please email this table to pglyco@ict.ac.cn to get the license.
4. Please let us know if you use pGlyco3 in your publication.

① Copy to clipboard ③ Import the license file

pFind Studio

a computational solution for mass spectrometry-based proteomics

License Application / pGlyco3

Please paste the registration form here:

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: ...
Activation code: ...

②

submit

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



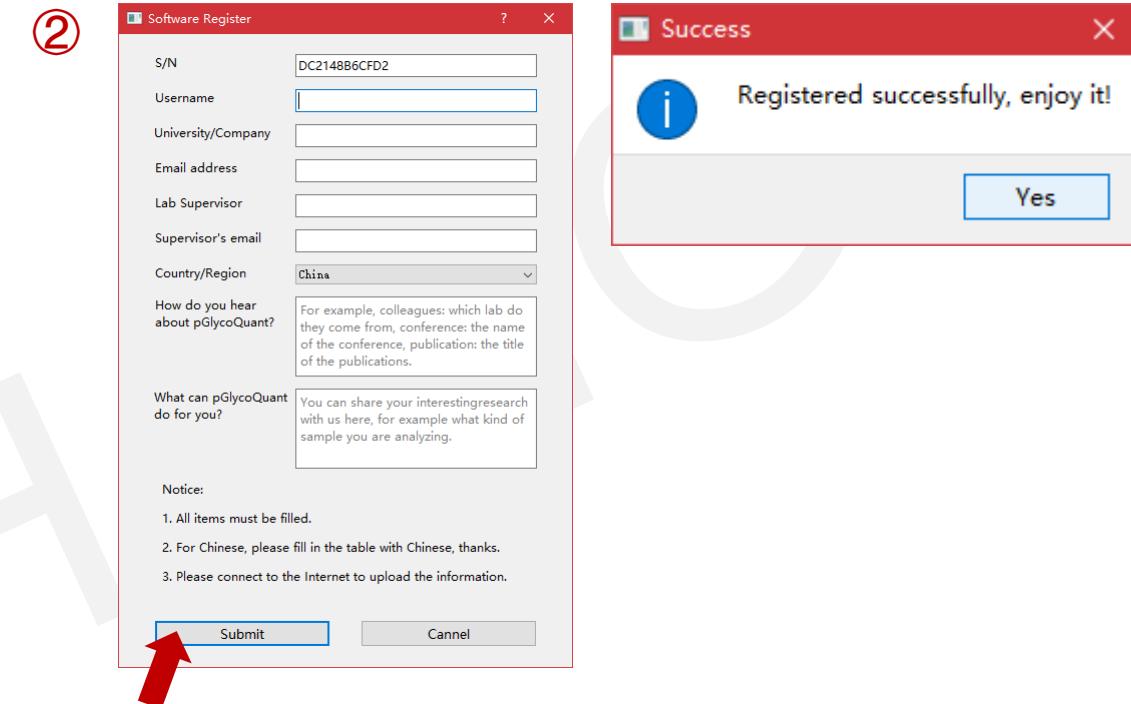
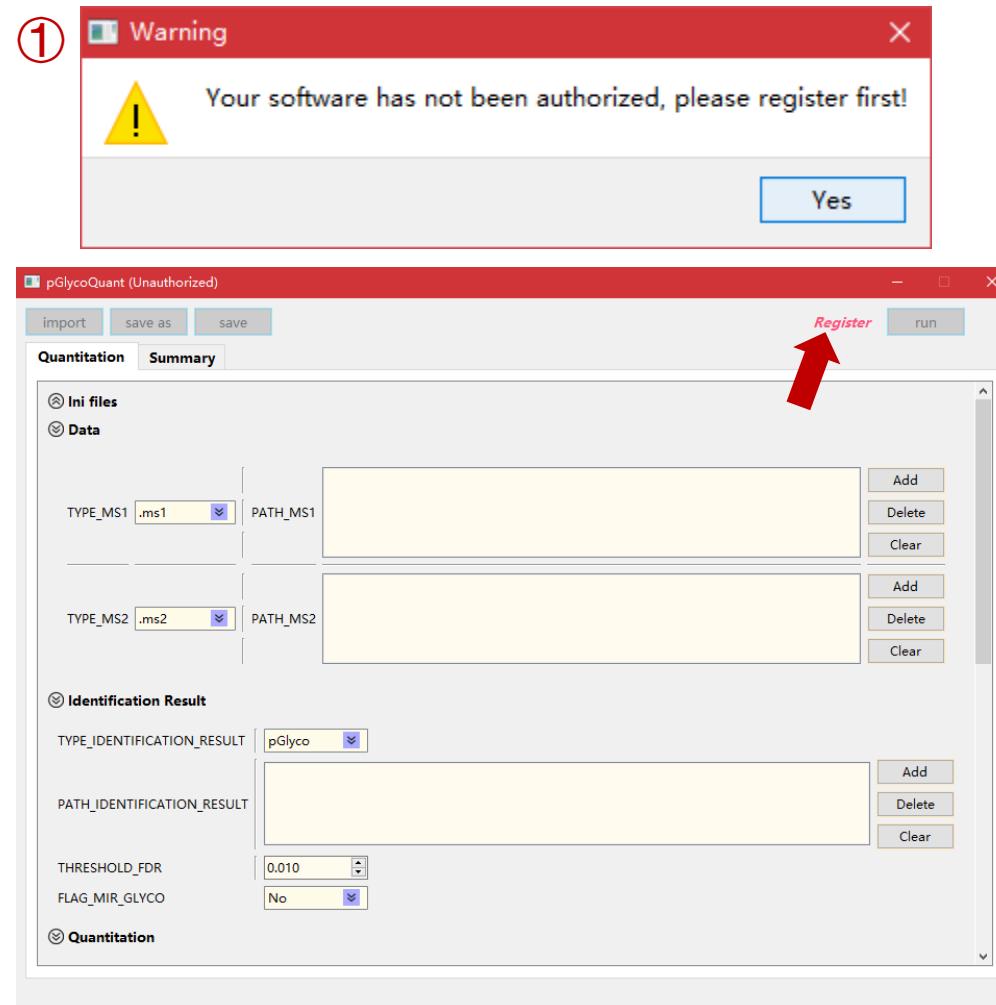
① Login <https://github.com/Power-Quant/pGlycoQuant/releases> and download the latest version at the top of the page.

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

② 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

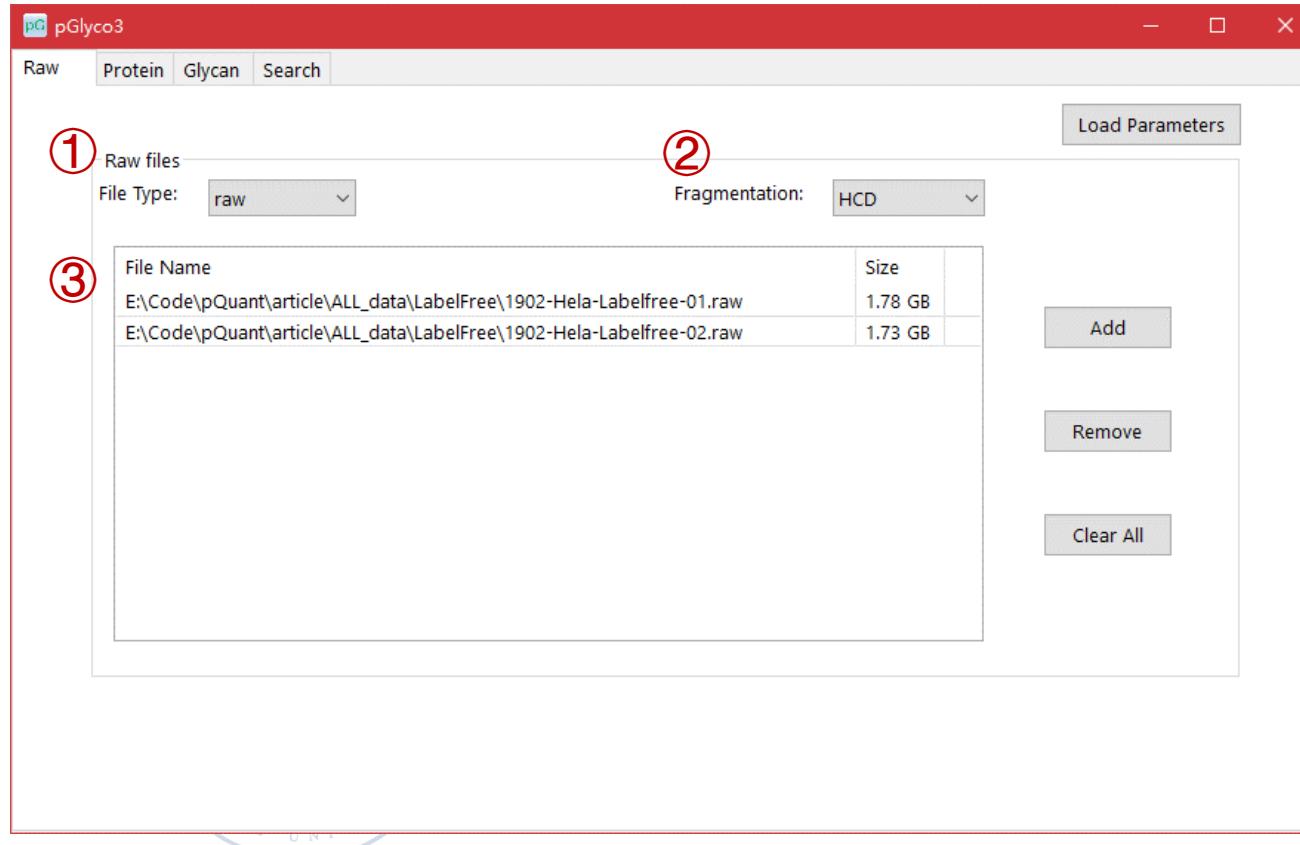
Manual for DDA Label Free Data



1 Identification with pGlyco

1.1 pGlyco identification

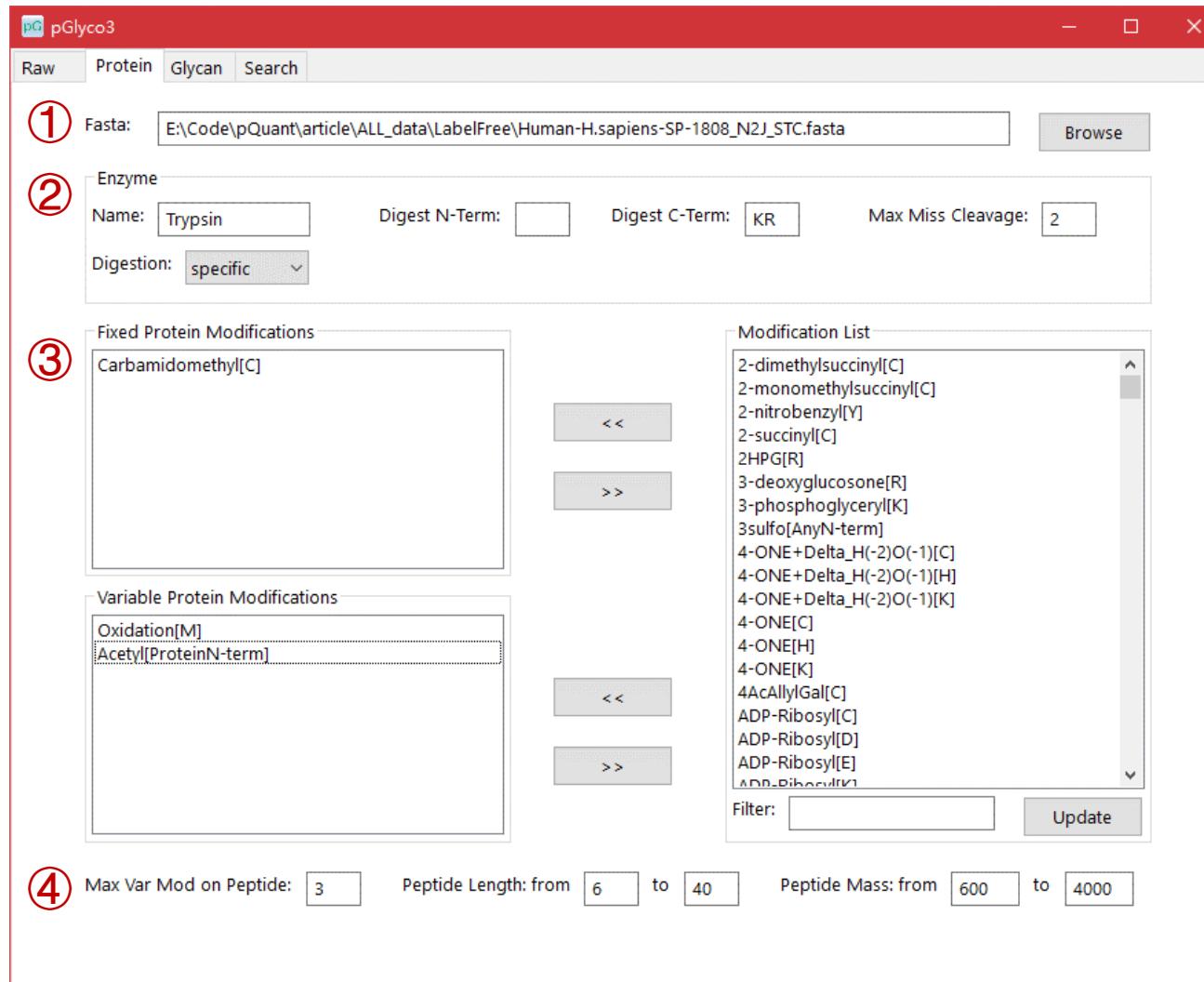
Label Free: pGlyco & pGlycoQuant



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

1 Identification with pGlyco

1.1 pGlyco identification

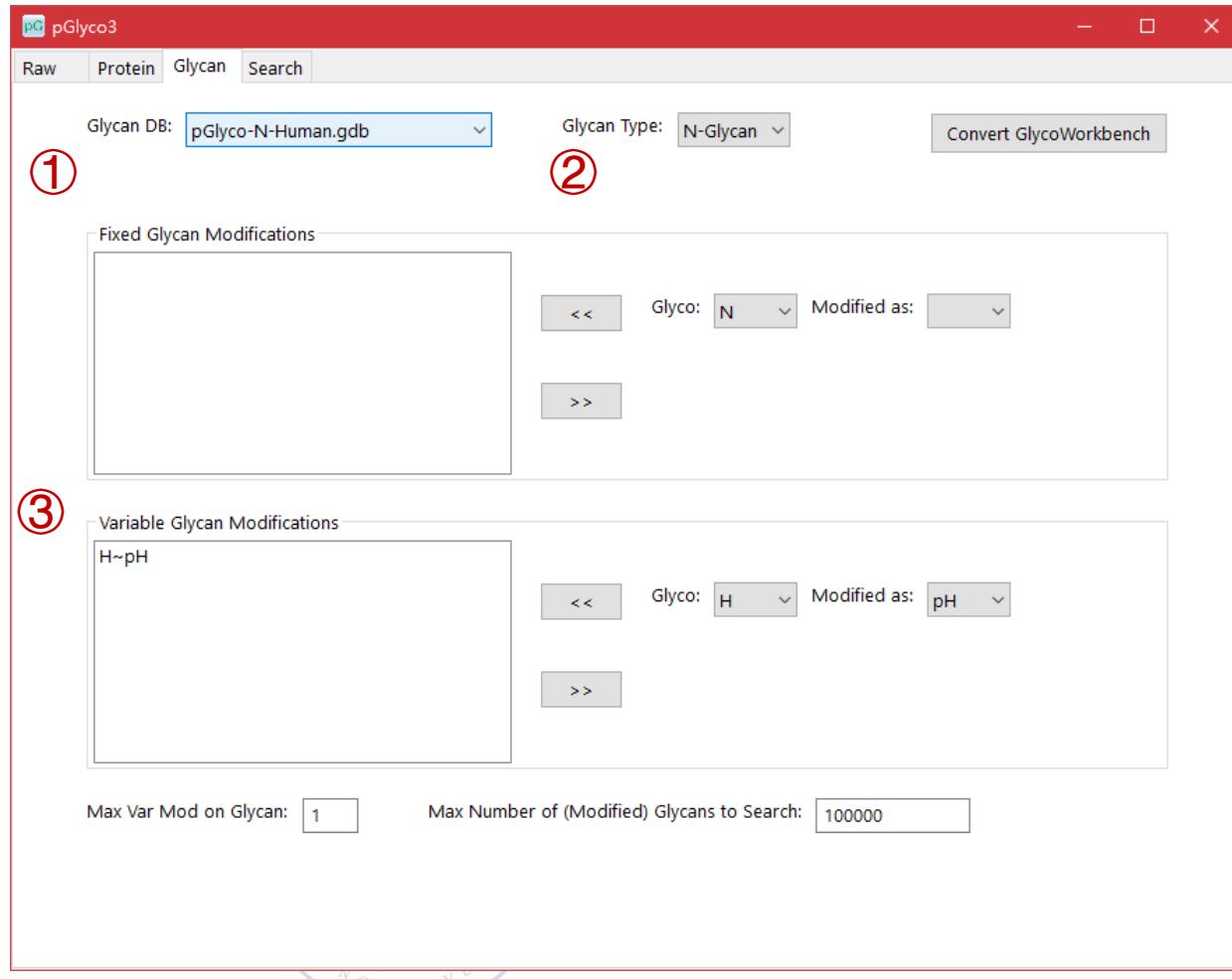


Label Free: pGlyco & pGlycoQuant

- ① Set the fasta database.
- 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: pGlyco & pGlycoQuant

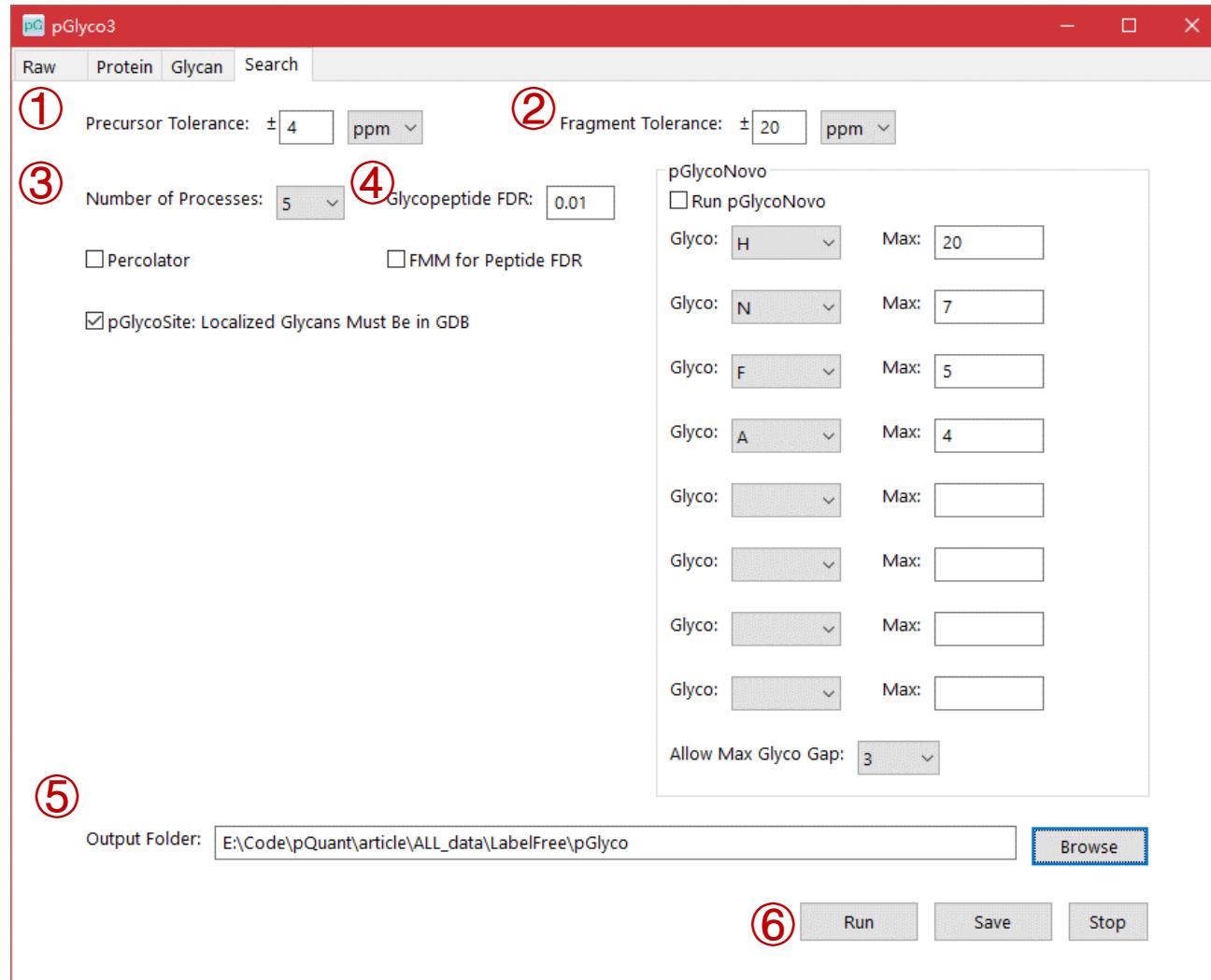


- ① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.
- ② Set the **Glycan Type** as **N-Glycan**.
- ③ Set the Glycan modification information.

1 Identification with pGlyco

1.1 pGlyco identification

Label Free: pGlyco & pGlycoQuant



- 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 command-line window with the following text:

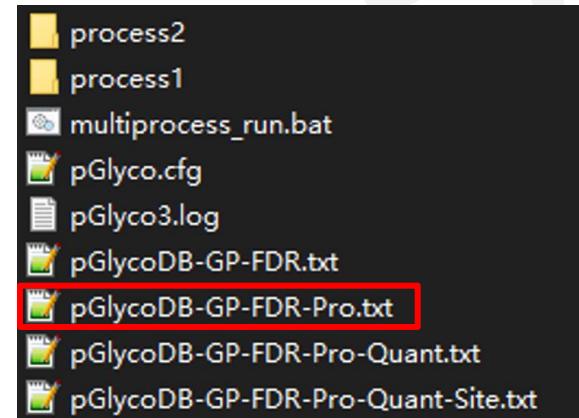
```
C:\Windows\system32\cmd.exe
E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\pGlyco.cfg
pParsePlusLog.txt
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3_0_build20210615\bin\rem ****
Process ID=25040: E:\Code\pQuant\article\software\pGlyco\pGlyco3_0_build20210615\bin\parse_raw.exe E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process1\pGlyco.cfg
Process ID=14252: E:\Code\pQuant\article\software\pGlyco\pGlyco3_0_build20210615\bin\parse_raw.exe E:\Code\pQuant\article\ALL_data\LabelFree\pGlyco\process2\pGlyco.cfg
Process ID=14252: pParsePlusLog()
Process ID=14252: pParsePlusLog() from pfind Studio
Process ID=14252: Email : pfind@ict.ac.cn
Process ID=14252: Website : http://pfink.ict.ac.cn
Process ID=14252: ****
Process ID=14252: The license will expire in 2100-1-1
Process ID=14252: pParse() INFO: pParse writes logs in E:\Code\pQuant\article\ALL_data\LabelFree\pParsePlusLog.txt
Process ID=14252: pParse() INFO: pParse reads configuration file E:\Code\pQuant\article\ALL_data\LabelFree\pParsePlusLog.txt
Process ID=14252: pParse() -01: check_activationcenter = 1
Process ID=14252: pParse() -02: co-solute = 1
Process ID=14252: pParse() -03: database = 1
Process ID=14252: pParse() -04: database =
Process ID=14252: pParse() -05: datapathl = E:\Code\pQuant\article\ALL_data\LabelFree\HeLa-Labelfree-02.ms1
Process ID=14252: pParse() -06: delete_msn = 0
Process ID=14252: pParse() -07: dia_mode_to_filter = 1
Process ID=14252: pParse() -08: dia_mode_to_filter_by_selectedMS2ScanSet = 1
Process ID=14252: pParse() -09: input_format = ms1
Process ID=14252: pParse() -10: ion_type = 1
Process ID=14252: pParse() -11: i_file = IPIV.txt
Process ID=14252: pParse() -12: isolation_width = 2.000000
Process ID=14252: pParse() -13: logfilepath = E:\Code\pQuant\article\ALL_data\LabelFree
Process ID=14252: pParse() -14: max_ms2count = 4
Process ID=14252: pParse() -15: mass_model = 4
Process ID=14252: pParse() -16: mass_threshold = -0.500000
Process ID=14252: pParse() -17: max_ms2count = 20.000000
Process ID=14252: pParse() -18: metolysate = 1
Process ID=14252: pParse() -19: output_msf = 1
Process ID=14252: pParse() -20: output_pf = 1
Process ID=14252: pParse() -21: outputpath =
Process ID=14252: pParse() -22: resultrate_window = 7.000000
```

1 Identification with pGlyco

1.2 identification results

①

```
选择C:\WINDOWS\system32\cmd.exe
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-01.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: 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: 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!
```



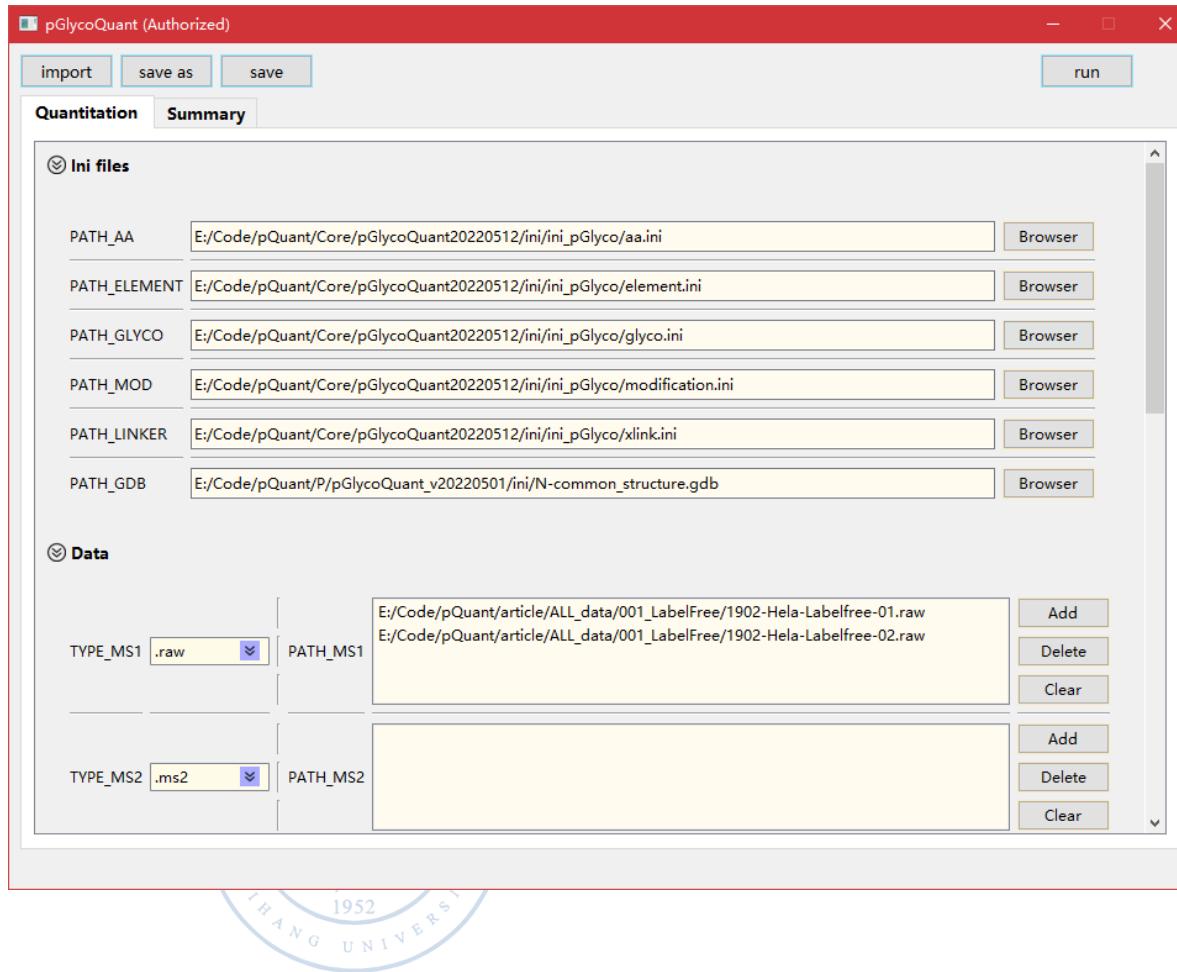
Label Free: pGlyco & pGlycoQuant

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

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

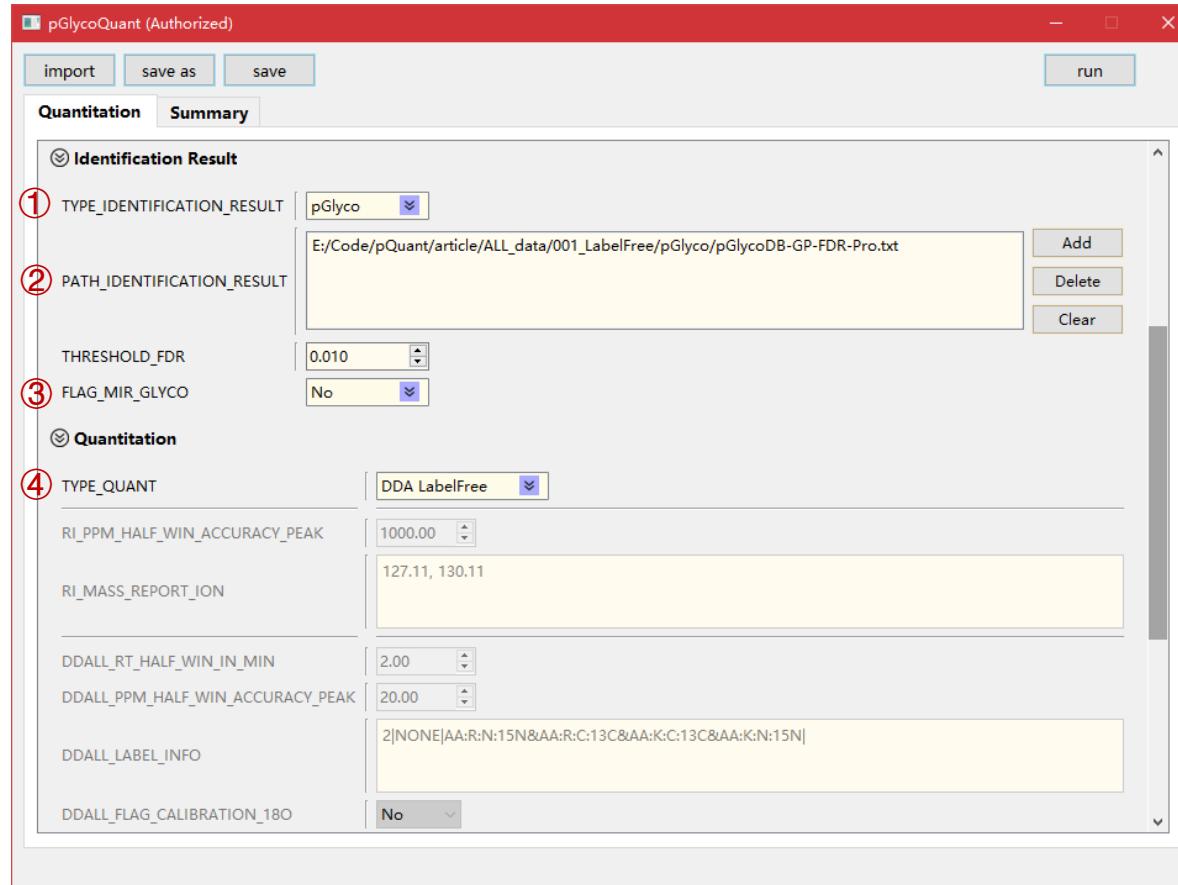
Label Free: pGlyco & pGlycoQuant



- 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

2.1 pGlycoQuant quantitation

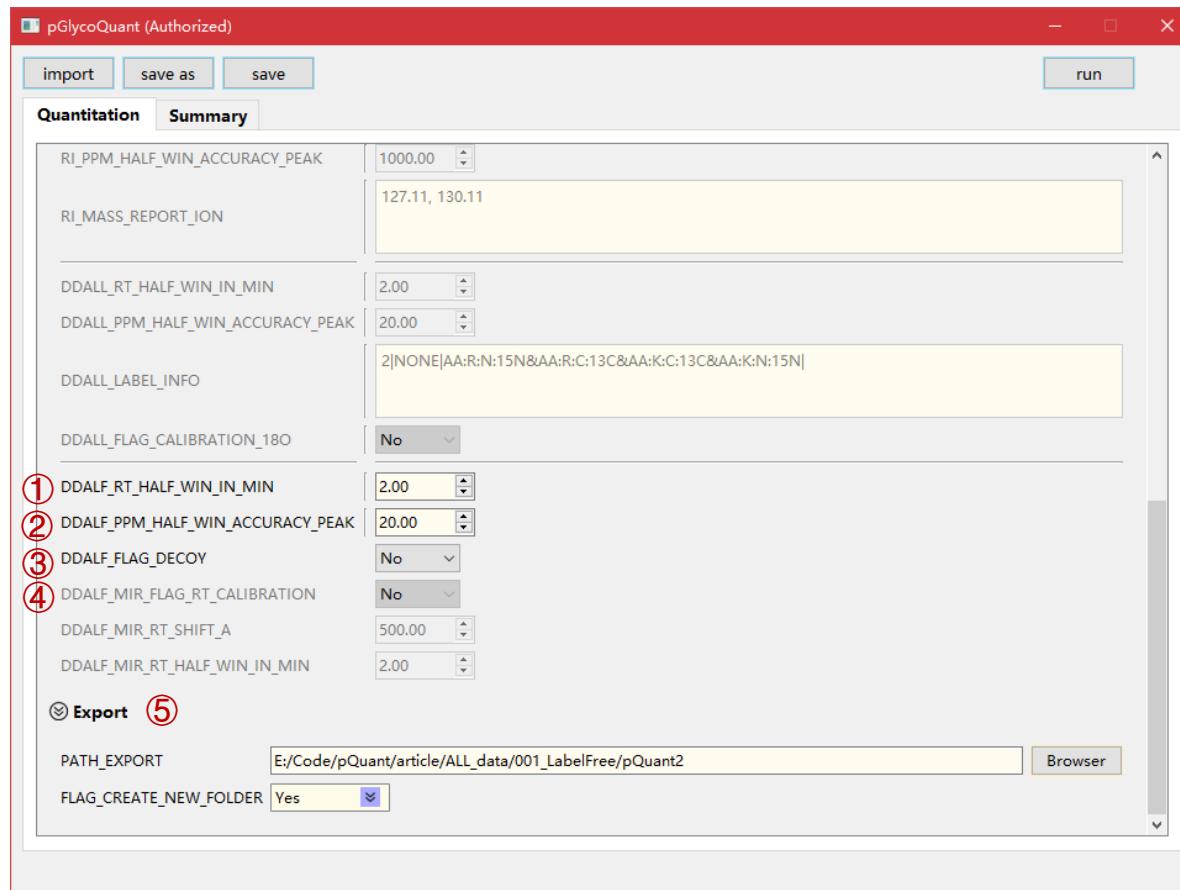


Label Free: pGlyco & pGlycoQuant

- ① Set **TYPE_IDENTIFICATION_RESULT** as **pGlyco**
(For other identification software results like Byonic and MSFagger 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

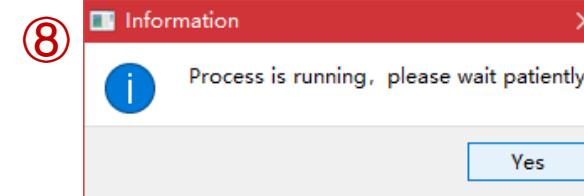
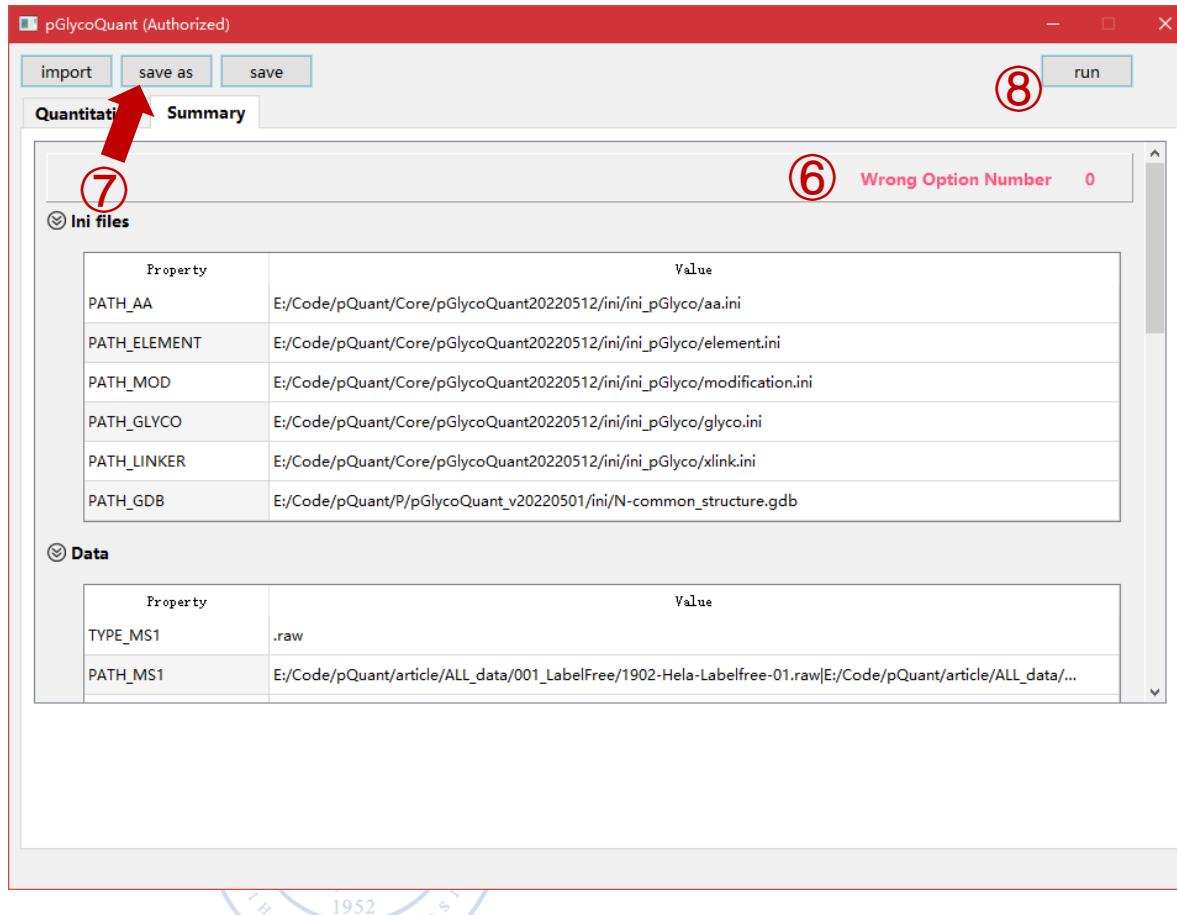


- ① Set **DDALF_RT_WIN_IN_MIN** as 2.00 minutes (default setting).
- ② Set **DDALF_PPM_HALF_WIN_ACCURACY_PEAK** as 20.00 ppm (default setting).
- ③ The **DDALF_FLAG_DECOY** can be set as **Yes** to use FQR only for Label Free data.
- ④ If **FLAG_MIR_GLYCO** is set as **Yes**:
DDALF_MIR_FLAG_RT_CALIBRATION 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.

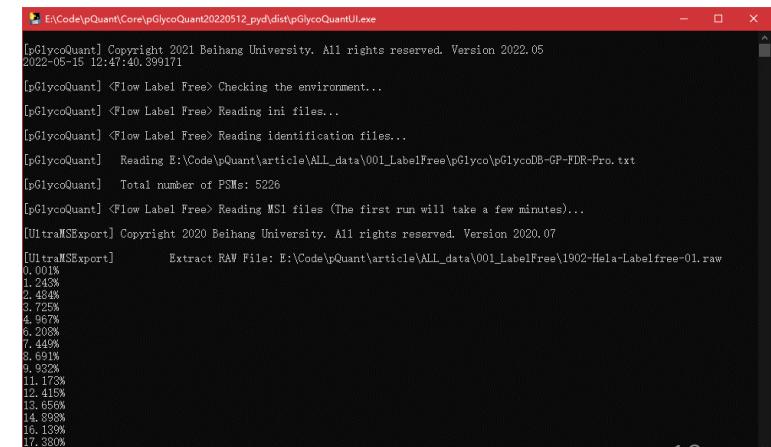
2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

Label Free: pGlyco & pGlycoQuant



- 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.



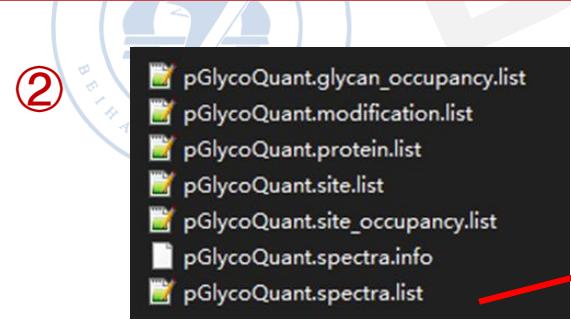
2 Quantitation with pGlycoQuant

2.2 quantitation results

Label Free: pGlyco & pGlycoQuant

①

```
E:\Code\pQuant\Core\pGlycoQuant20220512_pyd\dist\pGlycoQuantUI.exe
45.96%
53.61%
61.27%
68.92%
76.57%
84.23%
91.88%
99.54%
100.0%
[pGlycoQuant] <Function Quantitation> Getting decoy evidences for references...
0.03%
7.69%
15.34%
23.00%
30.65%
38.30%
45.96%
53.61%
61.27%
68.92%
76.57%
84.23%
91.88%
99.54%
100.0%
[pGlycoQuant] <Function Quantitation> Getting decoy evidences for samples...
0.03%
7.69%
15.34%
23.00%
30.65%
38.30%
45.96%
53.61%
61.27%
68.92%
76.57%
84.23%
91.88%
99.54%
100.0%
[pGlycoQuant] <Function FQR> Calculate FQR thresholds and filter quantitative results...
[pGlycoQuant] <Flow Label Free> Inferring...
[pGlycoQuant] Finished!
```



- 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

	Peptide	PerSpec	RawNameH	RT	Precursor/Precursor/Charge	Rank	Peptide	Mod	Peptide-Mt	GlycanH	Glycan-Cap	SiteSt	ClyD	GlyPep	GlyMass	GlySite	TotalScore	PepScore						
1	IAGLQLD	YHJYSGIPLDQFSS	HeLa1902	4911	1017.914	3099.956	1105.989	2	IAGLQLD	1899.975	7.2	0.0	H0N2	11421.0	1.0	0.000	1702.581	3	55.91601	20.17705				
2	IAGLQLD	YHJYSGIPLDQFSS	HeLa1902	15356	4121.43	3447.341	1149.785	3	IAGLQLD	1623.764	6.3	0.0	H0N2	13427.0	1.0	0.000	1823.574	10	35.33359	27.77551				
3	IATYHYAEGEEFHDV	1ATYHYAEGEEFHDV	HeLa1902	33563	9020.257	4087.664	1022.671	4	IATYHYAEGEEFHDV	2643.127	3.4	0.1	H0N2	3180.0	1.0	0.000	1444.534	1	13.39325	13.34168				
4	1DSLSUATNIK	1DSLSUATNIK	HeLa1902	29913	8036.366	2716.157	1358.582	2	1DSLSUATNIK	1175.627	7.2	0.0	H0N2	11421.0	1.0	0.000	1540.529	6	75.34538	81.6648				
5	1JCTSI5GDL2Carbam	1JCTSI5GDL2Carbam	HeLa1902	47834	1277.07	3440.505	147.507	3	1JCTSI5GDL2Carbam	1899.975	7.2	0.0	H0N2	11421.0	1.0	0.000	1540.529	1	82.41943	88.36935				
6	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	43617	11715.8	3591.438	1197.817	3	1YHJYSGIPLDQFSS	2212.96	6.2	0.0	H0N2	11463.0	1.0	0.000	1378.476	4	12.46524	12.25998				
7	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	39030	10487.29	2737.074	1369.041	2	1YHJYSGIPLDQFSS	2212.96	6.2	0.0	H0N2	11421.0	1.0	0.000	1702.581	3	53.74077	99.99352				
8	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	39030	10487.29	3220.006	1050.999	3	1YHJYSGIPLDQFSS	1603.023	6.2	0.0	H0N2	1025.0	1.0	0.000	1702.581	7	41.47777	39.39343				
9	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	23187	6261.761	2691.202	1346.105	2	1YHJYSGIPLDQFSS	988.619	6.0	0.0	H0N2	14636.0	1.0	0.000	1702.581	4	28.64275	19.13924				
10	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	34850	9365.015	3011.23	1508.119	1	1YHJYSGIPLDQFSS	1146.594	9.2	0.0	H0N2	10323.0	1.0	0.000	1864.634	3	30.92117	19.31954				
11	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	26333	7079.023	3634.581	1212.198	3	1YHJYSGIPLDQFSS	202.993	4.4	0.1	H0N2	3155.0	1.0	0.000	1606.587	10	15.81626	11.06153				
12	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	22591	6072.635	2626.13	1314.569	2	1YHJYSGIPLDQFSS	925.565	8.2	0.0	H0N2	10352.0	1.0	0.000	1702.581	7	35.83687	20.86558				
13	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	39030	10487.29	2737.074	1369.041	2	1YHJYSGIPLDQFSS	1198.548	7.2	0.0	H0N2	11421.0	1.0	0.000	1540.529	3	17.84072	10.33626				
14	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	26231	7051.652	3306.429	102.815	3	1YHJYSGIPLDQFSS	1765.998	7.2	0.0	H0N2	11421.0	1.0	0.000	1540.529	1	53.67543	54.75251				
15	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	39030	10487.29	3473.291	1158.02	3	1YHJYSGIPLDQFSS	1603.023	6.2	0.0	H0N2	1025.0	1.0	0.000	1702.581	4	28.64275	9.93845				
16	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	6744	1018.64	2193.459	455.455	2	1YHJYSGIPLDQFSS	4268.5	2.0	0.0	H0N2	1151.0	1.0	0.000	1702.581	4	28.64275	19.13924				
17	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	61866	16858.01	3410.352	1137.456	3	1YHJYSGIPLDQFSS	1748.829	5.3	0.0	H0N2	10431.0	1.0	0.000	1661.521	13	23.875	17.67687				
18	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	21632	5818.444	3770.521	1257.512	3	1YHJYSGIPLDQFSS	1710.783	5.4	0.1	H0N2	2373.0	1.0	0.000	2059.735	4	35.69245	19.07819				
19	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	37105	9972.603	3050.284	1525.646	2	1YHJYSGIPLDQFSS	10345.0	8.2	0.0	H0N2	10345.0	1.0	0.000	1702.581	2	4.28794	17.16471				
20	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	22540	6058.86	2808.154	936.723	3	1YHJYSGIPLDQFSS	1105.574	8.2	0.0	H0N2	10258.0	1.0	0.000	1702.581	2	15.52441	9.66117				
21	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	27278	7381.399	2896.238	1448.621	2	1YHJYSGIPLDQFSS	1198.652	8.2	0.0	H0N2	10920.0	1.0	0.000	1702.581	6	82.7849	85.88707				
22	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	30624	804.86	2470.107	102.817	7	1YHJYSGIPLDQFSS	769.308	6.2	0.0	H0N2	1025.0	1.0	0.000	1702.581	2	28.64275	18.03579				
23	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	39030	10487.29	1074.741	224.270	475.431	3	1YHJYSGIPLDQFSS	1228.66	7.3	0.1	H0N2	14340.0	1.0	0.000	1702.581	4	32.08625	27.71136			
24	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	22522	6054.02	2626.13	1314.569	3	1YHJYSGIPLDQFSS	925.565	8.2	0.0	H0N2	10352.0	1.0	0.000	1702.581	7	36.49143	24.66389				
25	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	37906	10185.56	3961.171	1321.241	3	1YHJYSGIPLDQFSS	209.072	9.2	0.0	H0N2	14542.0	1.0	0.000	1864.634	6	17.41539	9.41301				
26	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	41167	11058.03	2611.214	871.0763	3	1YHJYSGIPLDQFSS	157.288	3.2	0.0	H0N2	1038.375	1.0	0.000	1702.581	2	31.04382	29.92218				
27	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	38707	10400.71	4033.613	1345.209	3	1YHJYSGIPLDQFSS	2166.977	9.2	0.0	H0N2	14673.0	1.0	0.000	1864.634	4	39.80104	31.76965				
28	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	10247	2748.709	2598.048	166.6875	3	1YHJYSGIPLDQFSS	1219.557	6.2	0.0	H0N2	11510.0	1.0	0.000	1378.476	4	51.20717	44.77136				
29	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	47842	1285.49	4106.816	1027.459	4	1TDEQVQREEAQIQLQD	2728.338	6.2	0.0	H0N2	11510.0	1.0	0.000	1378.476	19	57.50054	70.20237				
30	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	39030	10487.29	1074.741	224.270	475.431	5	1YHJYSGIPLDQFSS	1198.652	8.2	0.0	H0N2	1025.0	1.0	0.000	1702.581	5	32.08625	18.03579			
31	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	16731	4492.785	2593.028	102.817	2	1FISSSSLEEK	12145.64	5.2	0.0	H0N2	14983.0	1.0	0.000	1378.476	5	53.50094	60.08892				
32	1YHJYSGIPLDQFSS	1YHJYSGIPLDQFSS	HeLa1902	52885	14211.46	4233.873	1059.224	4	1LPSTIVVEPGVLYGFTV	2651.293	9.2	0.0	H0N2	10258.0	1.0	0.000	1702.581	3	25.87243	23.33618				

2 Quantitation with pGlycoQuant

2.2 quantitation results --- MIR results

Label Free: pGlyco & pGlycoQuant

	AL	AM	AN	AO	AP
1	Genes	ProSites	Supp_Info	Empty_Sep	Intensity(2CIR)
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(N(H))))(H(N(H)))))		28256694
2762	IGHG1	180	H(5)N(4)A(1)_(N(N(H(H(N(H))))(H(N(H(A))))))		1417575
2763	IGHG1	180	H(5)N(4)A(2)_(N(N(H(H(N(H))))(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
2766	IGHG1	180	H(5)N(4)A(2)_(N(N(H(H(N(H))))(H(N(H(A))))))		66666667

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.



Byonic Result & pGlycoQuant

Manual for DDA Label Free Data



1 Get Byonic Results

①

 1902-Hela-Labelfree-Byonic-1.spectra.txt
 1902-Hela-Labelfree-Byonic-2.spectra.txt

②



Comment
1902-hela-labelfree-01.406.406.2
1902-hela-labelfree-01.632.632.2
1902-hela-labelfree-01.708.708.2
1902-hela-labelfree-01.784.784.2
1902-hela-labelfree-01.864.864.2
1902-hela-labelfree-01.932.932.2
1902-hela-labelfree-01.3500.3500.2
1902-hela-labelfree-01.4432.4432.3
1902-hela-labelfree-01.5474.5474.2

Correct

Label Free: Byonic Result & pGlycoQuant

① Find the identification result in “objs” folder of Byonic result file folder which is named “**output.spectra.txt**”.

For our provided testing files, you can find them in the test folder directly. They were renamed from the “**output.spectra.txt**”).

② Check the filenames of **raw data** and **the related filenames in identification results**, make sure they are the same.

 1902-Hela-Labelfree-01.raw
 1902-Hela-Labelfree-02.raw

Wrong

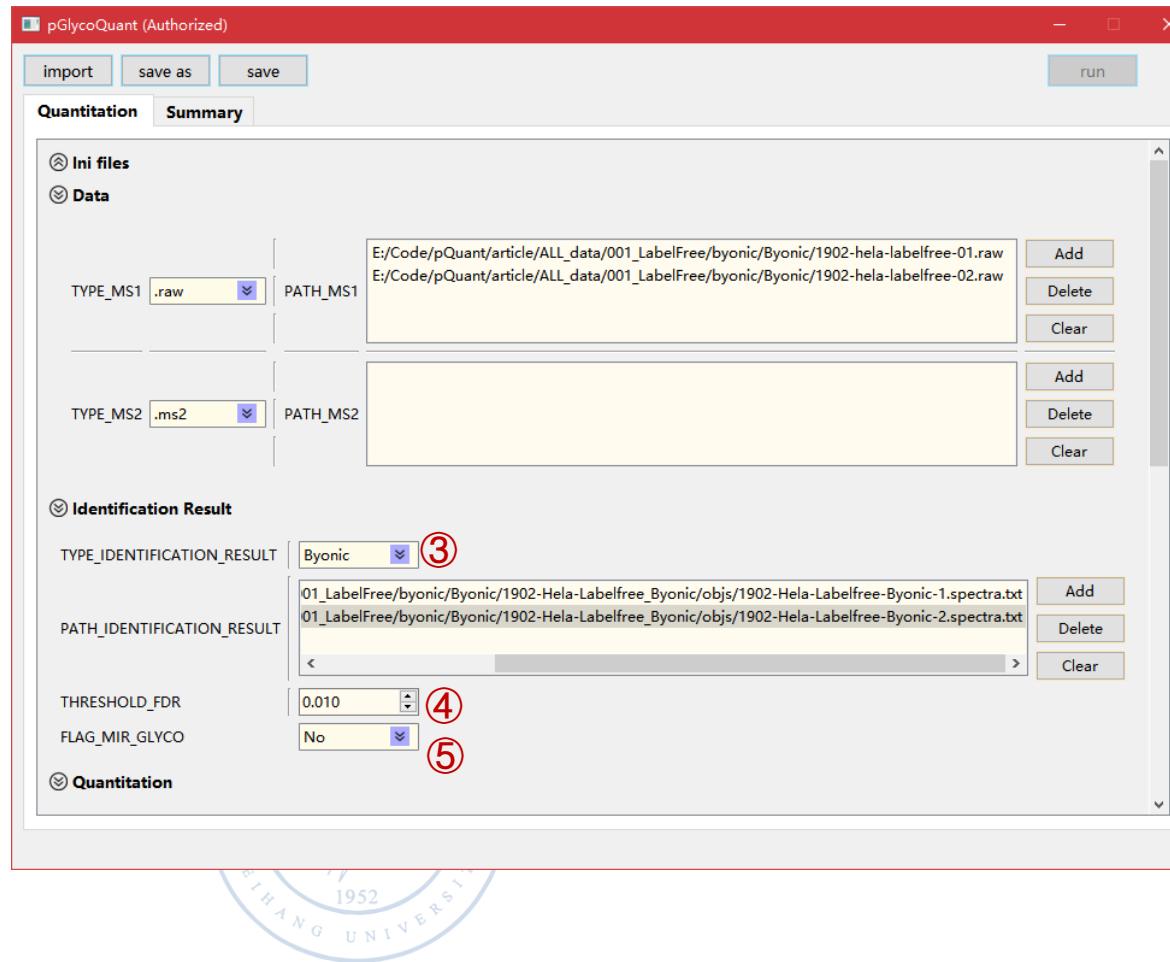
Which may cause this
ERROR:

```
[pGlycoQuant] <Flow Label Free> Quantifying...
[pGlycoQuant] <Function Quantitation> Getting evidences for references...
[pGlycoQuant] <Function Quantitation> Getting evidences for samples...
Traceback (most recent call last):
  File "MSFunctionQuant.py", line 1613, in quantForMulProcess
  File "MSFunctionQuant.py", line 828, in __captainUpdateSamplesForMulProcess
  File "MSFunctionEvidence.py", line 1183, in fillEvidence2
AttributeError: 'list' object has no attribute 'MID_RT'
Traceback (most recent call last):
  File "MSFunctionQuant.py", line 1613, in quantForMulProcess
  File "MSFunctionQuant.py", line 828, in __captainUpdateSamplesForMulProcess
  File "MSFunctionEvidence.py", line 1183, in fillEvidence2
AttributeError: 'list' object has no attribute 'MID_RT'
Traceback (most recent call last):
  File "pGlycoQuant.py", line 23, in <module>
  File "MSStaff.py", line 34, in start
  File "MSStaff.py", line 78, in __captainRunFlow
  File "MSFlow.py", line 81, in run
  File "MSTask.py", line 594, in workForMulProcess
AttributeError: 'list' object has no attribute 'DIS_ISO MOZ_CLC'
[29636] Failed to execute script 'pGlycoQuant' due to unhandled exception!
```

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

Label Free: Byonic Result & pGlycoQuant



① Ensure that the ini file paths are valid.

You can set the ③ **TYPE_QUANT** first, and the ini files will be set automatically.

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

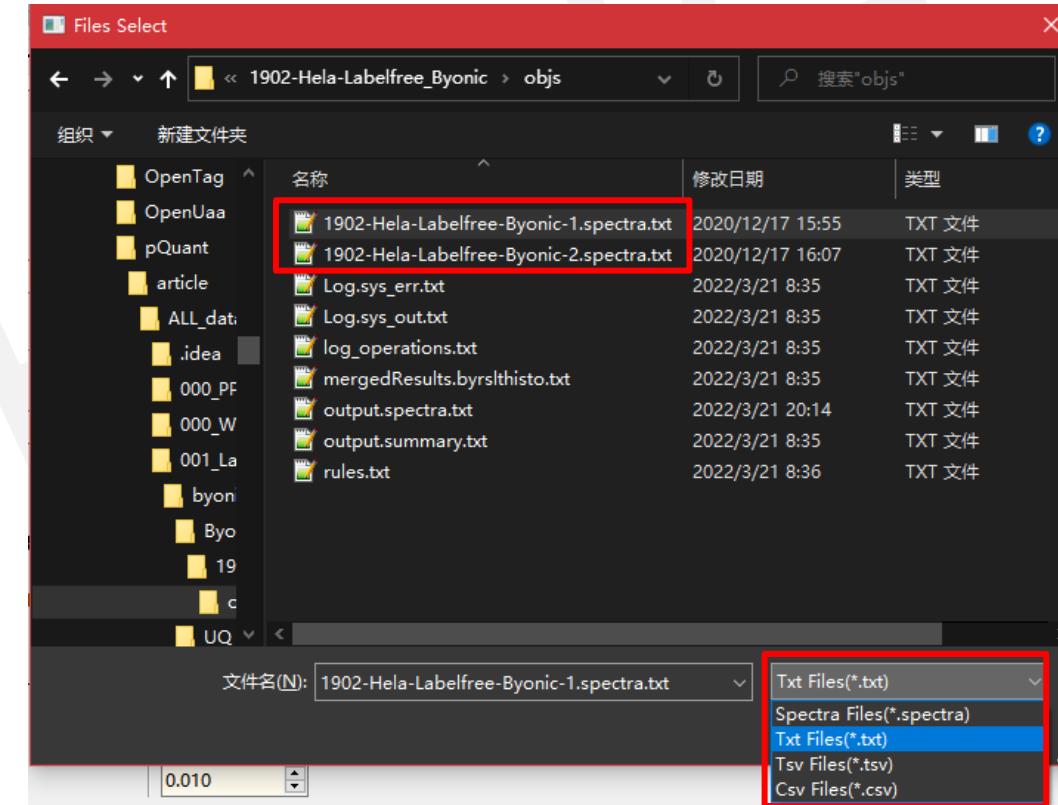
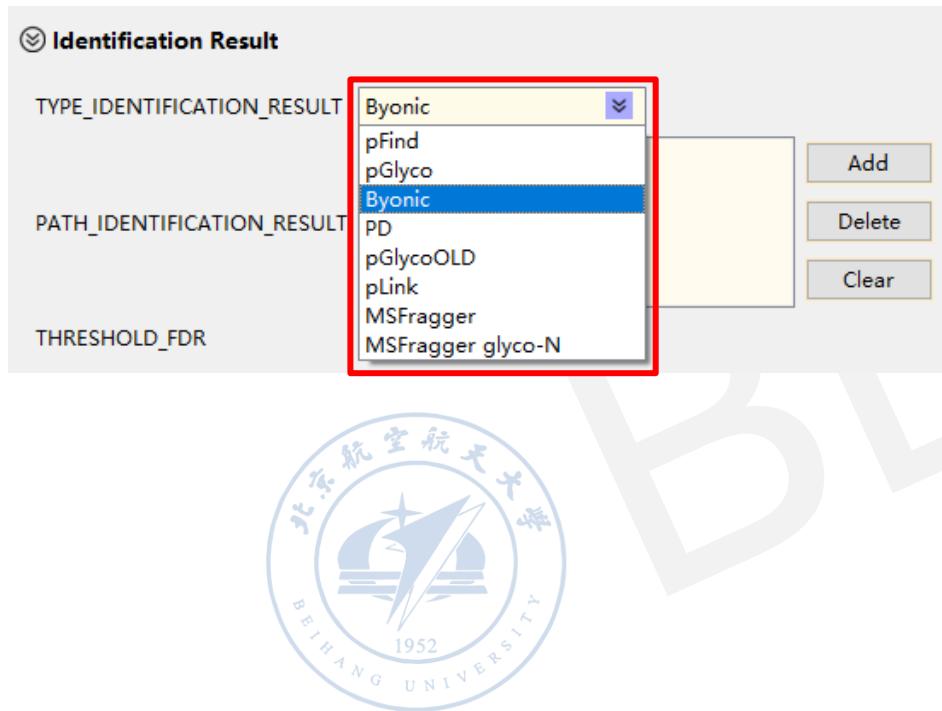
③ Set **TYPE_IDENTIFICATION_RESULT** as **Byonic** and fille the related identification results.

④ Set **FDR** as **0.01**.

⑤ Set the **FLAG_MIR_GLYCO** as **No**. (Only for pGlyco Label Free data now.)

Choose the related type of identification result for quantitation

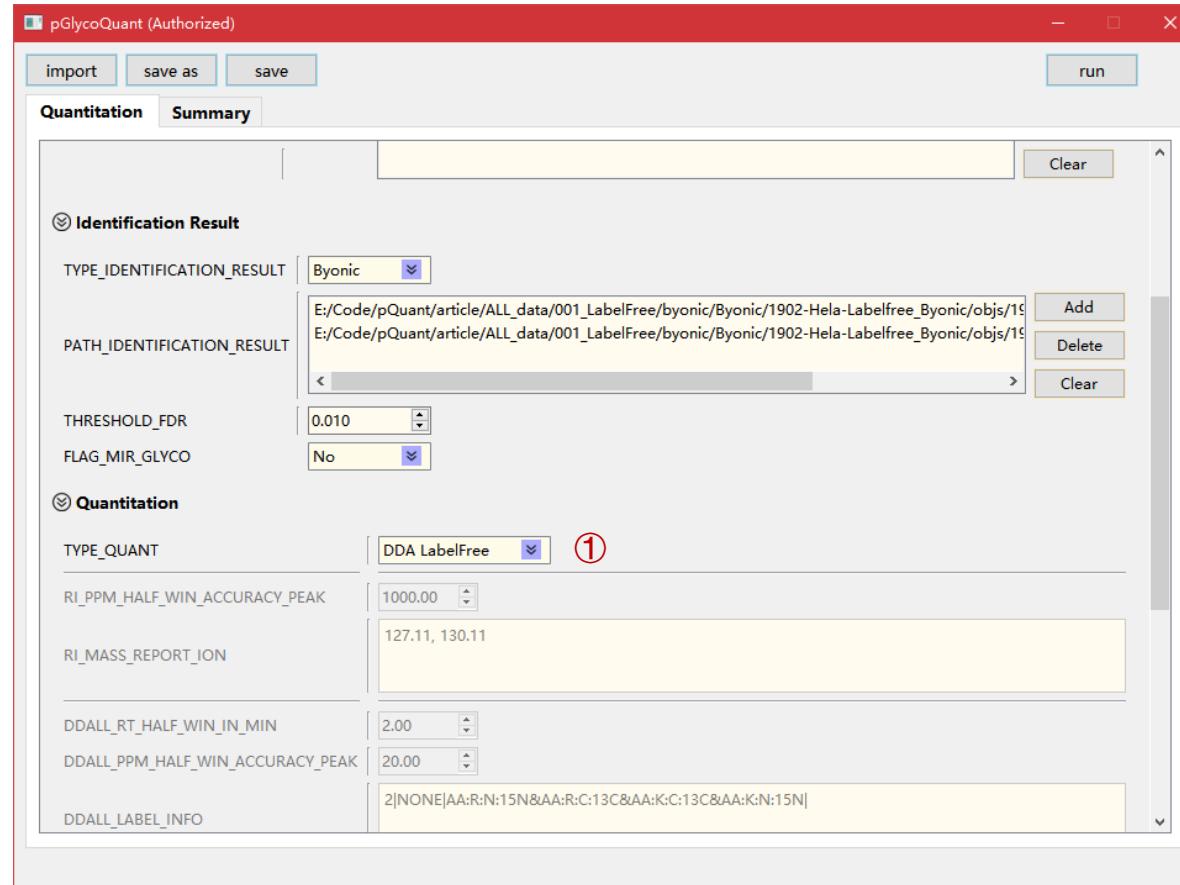
Label Free: Byonic Result & pGlycoQuant



2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

Label Free: Byonic Result & pGlycoQuant



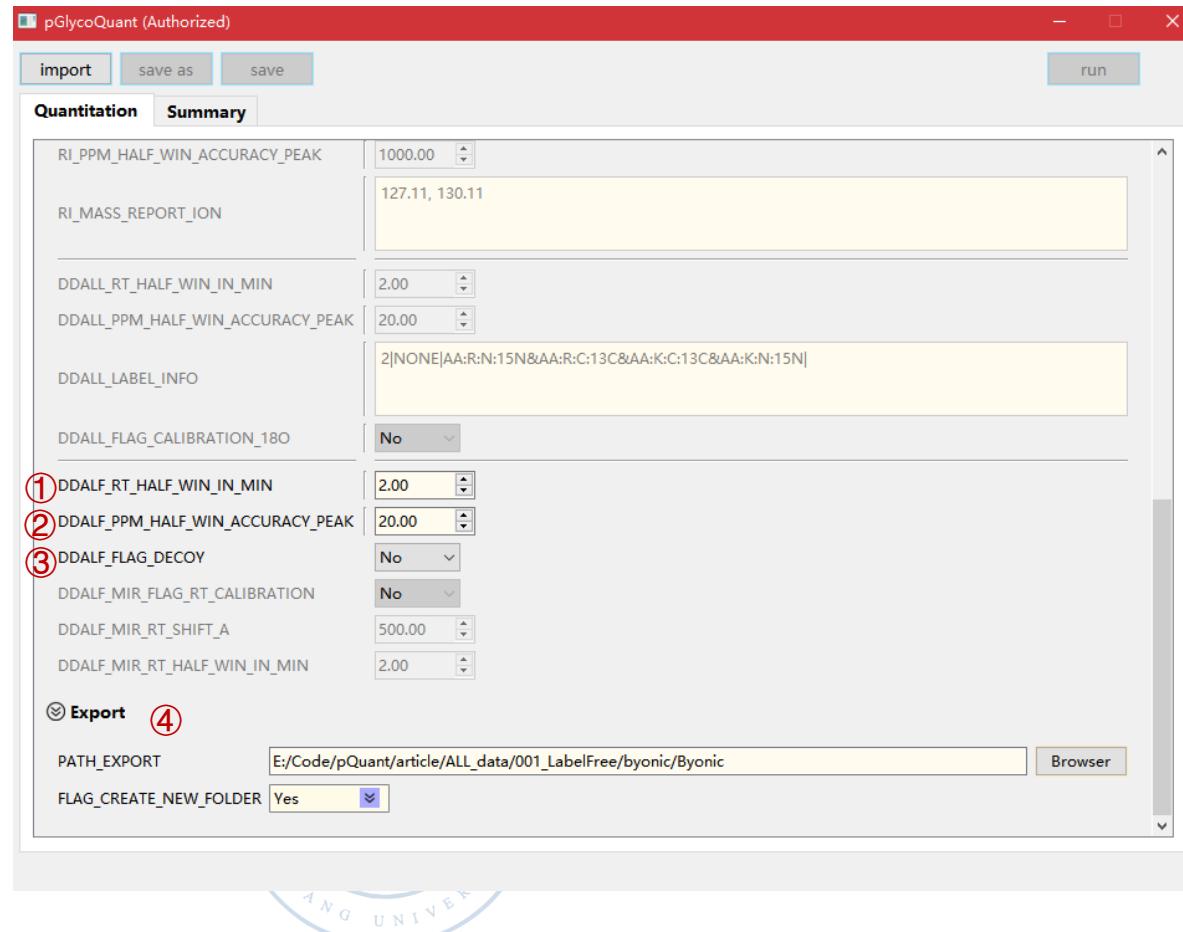
① Set the **TYPE_QUANT** as **DDA LabelFree**.



2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

Label Free: Byonic Result & pGlycoQuant

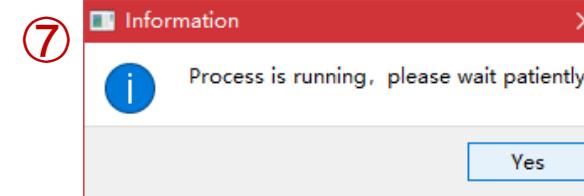
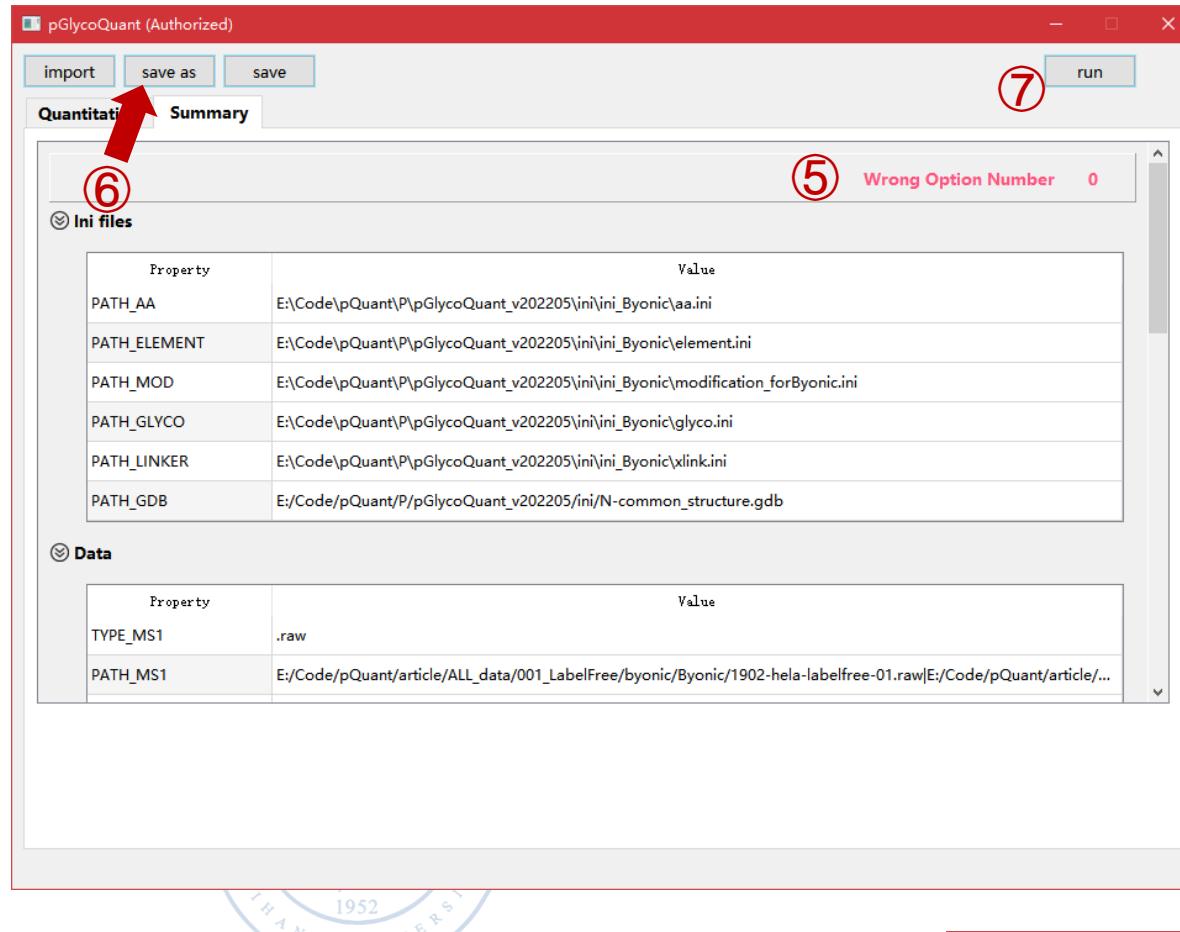


- ① Set **DDALF_RT_WIN_IN_MIN** as 2.00 minutes (default setting).
- ② Set **DDALF_PPM_HALF_WIN_ACCURACY_PEAK** as 20.00 ppm (default setting).
- ③ Set the **DDALF_FLAG_DECOY** as **No**.
- ④ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

Label Free: Byonic Result & pGlycoQuant



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

The command-line interface shows the execution of the pGlycoQuant software. It displays copyright information, environment checking, reading ini files, identification files, and spectra. It also shows the total number of PSMs (3363 and 7015), reading MS1 files, and extracting raw files. The UItraMSEexport version is also mentioned.

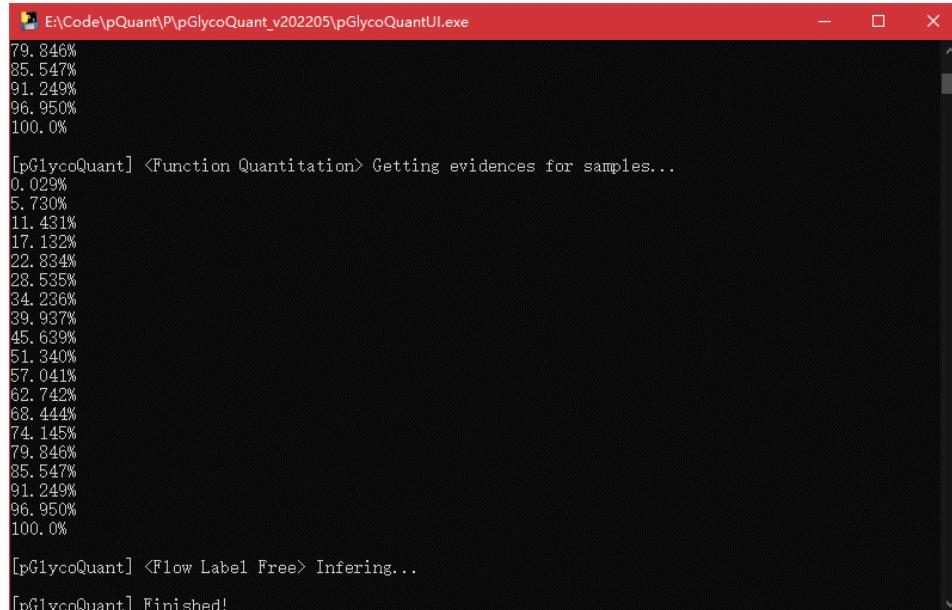
```
[pGlycoQuant] Copyright 2021 Beihang University. All rights reserved. Version 2022.05  
2022-05-20 23:45:35.050549  
[pGlycoQuant] <Flow Label Free> Checking the environment...  
[pGlycoQuant] <Flow Label Free> Reading ini files...  
[pGlycoQuant] <Flow Label Free> Reading identification files...  
[pGlycoQuant] Reading E:\Code\pQuant\article\ALL_data\001_LabelFree\byonic\1902-Hela-Labelfree-Byonic-1.spectra.txt  
[pGlycoQuant] Total number of PSMs: 3363  
[pGlycoQuant] Reading E:\Code\pQuant\article\ALL_data\001_LabelFree\byonic\1902-Hela-Labelfree_Byonic\objs\1902-Hela-Labelfree-Byonic-2.spectra.txt  
[pGlycoQuant] Total number of PSMs: 7015  
[pGlycoQuant] <Flow Label Free> Reading MS1 files (The first run will take a few minutes)...  
[UItraMSEexport] Copyright 2020 Beihang University. All rights reserved. Version 2020.07  
[UItraMSEexport] Extract RAW File: E:\Code\pQuant\article\ALL_data\001_LabelFree\byonic\1902-hela-labelfree-01.raw  
0.00%  
1.24%  
2.48%  
3.72%
```

2 Quantitation with pGlycoQuant

2.2 quantitation results

Label Free: Byonic Result & pGlycoQuant

①



② The completed information.

③ The quantitation results. Please open the files with Excel.



④

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Spectrum	Comment	Peptide	Mass (M+H) Observed	One-Off	Err (ppm)	Obs m/z	Prot#	Rank	NumUniq	Protein	Na/Position	Cleavage	Score	Delta	DeltaMo
2	09211.2	1902-hela-KFAASGP	1399.56	1399.561		1.1	700.2841	5535	1536	1	>sp P0C64	476	CRagged	273.73	255.13	255
3	02500.2	1902-hela-R.NI+1216	1705.701	1705.702		0.9	653.3548	14291	19	59	>sp P0555	269	Specific	317.12	19.23	19
4	02613.2	1902-hela-K.NI+1378	1853.763	1853.766		1.3	927.3865	523	235	25	>sp Q6YH+	279	Specific	279.57	279.57	279
5	02741.2	1902-hela-K.NI+1216	1837.725	1837.727		1	919.3672	2436	849	23	>sp P1515;	218	Specific	397.07	89.64	89
6	02809.2	1902-hela-K.NI+1216	1837.725	1837.727		0.8	919.3671	2436	849	23	>sp P1515;	218	Specific	413.61	89.22	89
7	02815.2	1902-hela-K.NI+1216	1837.725	1837.727		0.8	919.3671	2436	849	23	>sp P1515;	218	Specific	330.26	81.88	81
8	02879.2	1902-hela-K.NI+1216	1837.725	1837.727		0.2	919.3665	2436	849	23	>sp P1515;	218	Specific	265.81	63.98	63
9	03214.2	1902-hela-R.NLEK[N]+	1809.822	1809.824		1.2	905.4158	7426	7	69	>sp P0760;	422	Specific	339.01	96.25	96
10	03281.2	1902-hela-R.NLEK[N]+	1809.822	1809.823		0.6	905.4153	7426	7	69	>sp P0760;	422	Specific	527.5	196.52	196
11	03345.2	1902-hela-R.NLEK[N]+	1809.822	1809.823		0.6	905.4153	7426	7	69	>sp P0760;	422	Specific	487.49	170.14	170
12	05038.2	1902-hela-R.NI+1216	1776.738	1776.739		0.7	988.8732	2436	849	23	>sp P1515;	105	Specific	292.69	21.01	21
13	05920.2	1902-hela-R.YETTN[-]	1793.732	1793.733		0.7	897.3704	16471	2	91	>sp P1347;	54	Specific	280.88	81.67	81
14	05985.2	1902-hela-R.YETTN[-]	1793.732	1793.734		0.9	897.3705	16471	2	91	>sp P1347;	54	Specific	347.38	134.8	134
15	06056.2	1902-hela-R.YETTN[-]	1793.732	1793.734		0.8	897.3704	16471	2	91	>sp P1347;	54	Specific	366.58	125.96	125
16	06184.2	1902-hela-R.YETTN[-]	1631.679	1631.681		1.2	816.3442	16471	2	91	>sp P1347;	54	Specific	380.63	132.35	132
17	07105.2	1902-hela-KLDDNN[-]	1770.764	1770.763		-0.3	885.8853	7426	7	69	>sp P0760;	328	Specific	285.28	70.37	70
18	10186.2	1902-hela-R.IIN[-]+109	1857.832	1857.833		0.4	929.4201	16471	2	91	>sp P1347;	274	Specific	269.19	45.63	45
19	12181.2	1902-hela-R.NI+1216	1863.774	1863.774		0.2	932.3909	5958	459	16	>sp P5045-	125	Specific	385.25	156.52	156
20	12242.2	1902-hela-R.NI+1216	1863.774	1863.776		1.1	932.3916	5958	459	16	>sp P5045-	125	Specific	322.69	139.36	139
21	01450.2	1902-hela-K.NI+1709	2104.827	2104.83		1	1052.918	3402	254	13	>sp P0015	397	Specific	230.76	230.76	230
22	02533.2	1902-hela-K.NI+1378	1999.778	1999.781		1.6	1000.394	2436	849	23	>sp P1515;	218	Specific	414.97	85.89	85
23	02540.2	1902-hela-K.NI+1378	2061.779	2061.781		0.7	1038.94	13952	1391	25	>sp Q8WVHH-	246	Specific	346.33	91.19	91
24	02555.2	1902-hela-K.NI+1540	2015.816	2015.82		1.9	1008.414	523	235	25	>sp Q8WVHH-	270	Specific	319.63	319.63	319
25	03007.2	1902-hela-K.NI+1378	1899.726	1899.728		1	950.3675	1392	1391	25	>sp Q8WVWH-	2485	Specific	334.82	92.31	92
26	02646.3	1902-hela-K.HGSN+[E]	2122.866	2122.868		0.4	708.2942	332	414	9	>sp Q1281	579	Specific	379.18	172.51	172
27	02714.3	1902-hela-K.HGSN+[E]	2123.867	2123.869		0.7	924.2943	332	414	9	>sp Q1281	579	Specific	309.33	100.38	100
28	02931.2	1902-hela-R.NI+1540	2043.822	2043.823		0.4	1022.415	13605	806	29	>sp P0806-	135	Specific	254.81	254.81	254
29	03010.2	1902-hela-R.NI+1540	2043.822	2043.825		1.3	1022.416	13605	806	29	>sp P0806-	135	Specific	254.45	254.45	254
30	03155.2	1902-hela-R.NLEK[N]+	1971.875	1971.878		1.6	986.4427	7426	7	69	>sp P0760;	422	Specific	494.51	185.47	185
31	03P13.2	1902-hela-R.NI+FKNI[-]	1971.875	1971.878		1.4	986.4425	7426	7	69	>sp P0760;	422	Specific	504.32	187.17	187

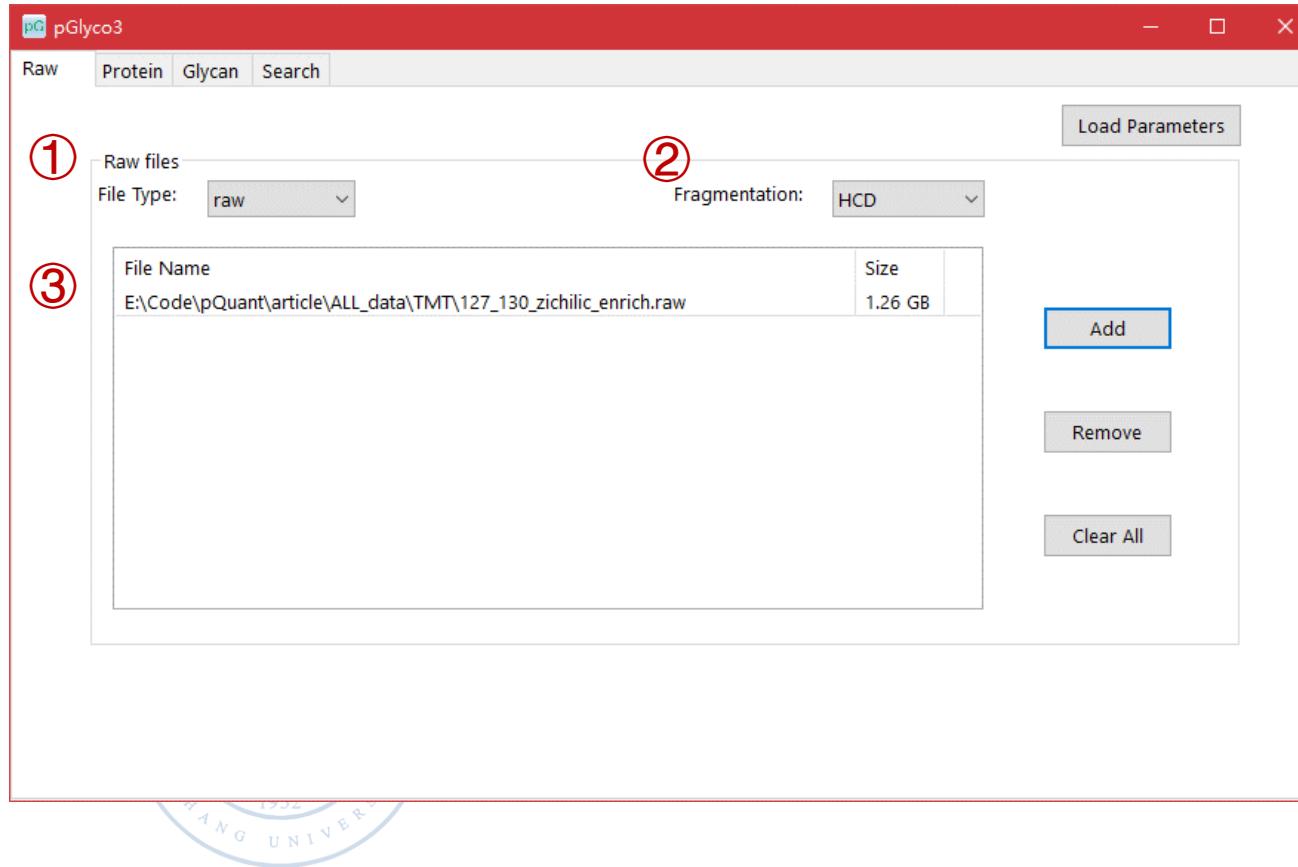
pGlyco & pGlycoQuant

Manual for TMT Data



1 Identification with pGlyco

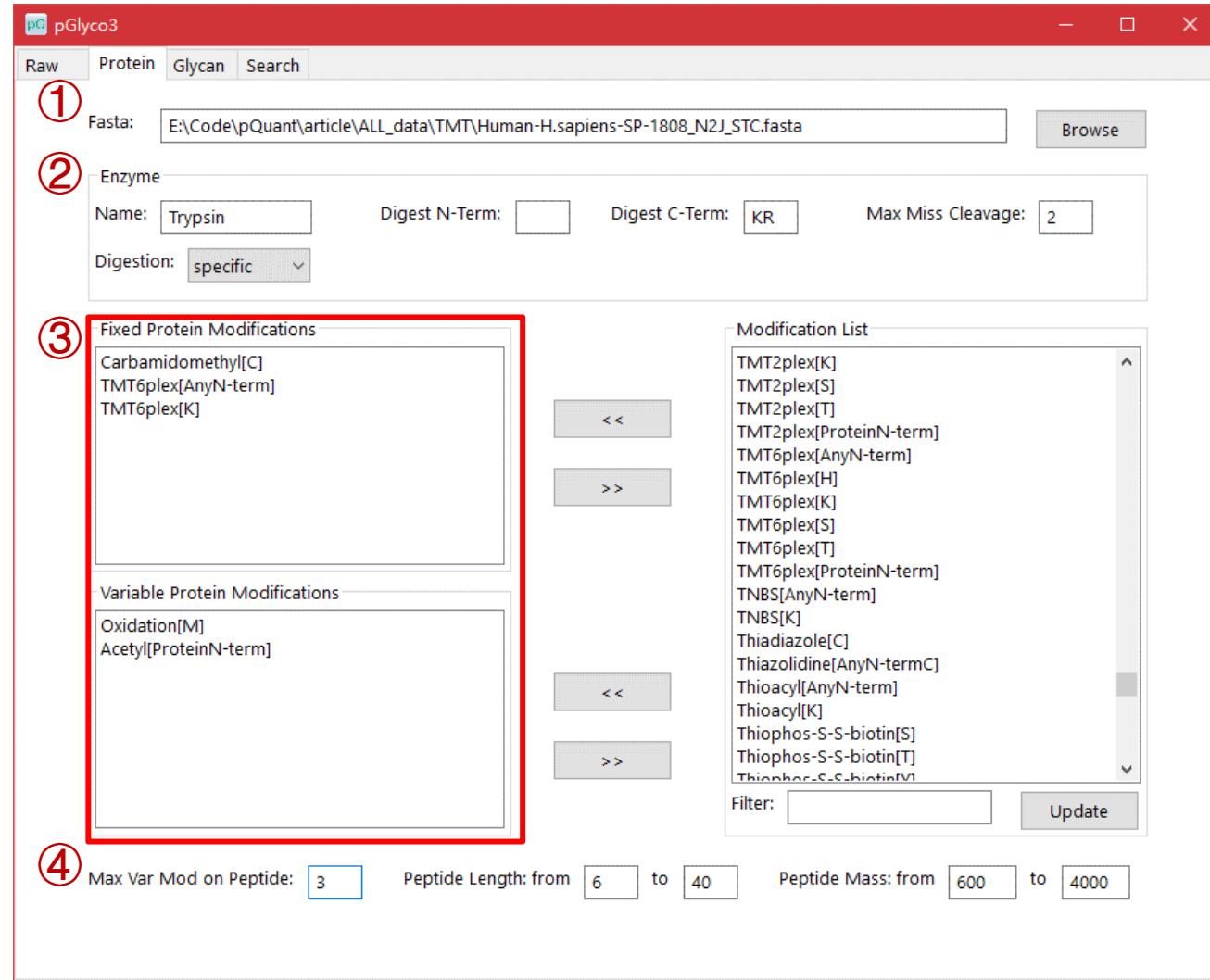
TMT



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

1 Identification with pGlyco

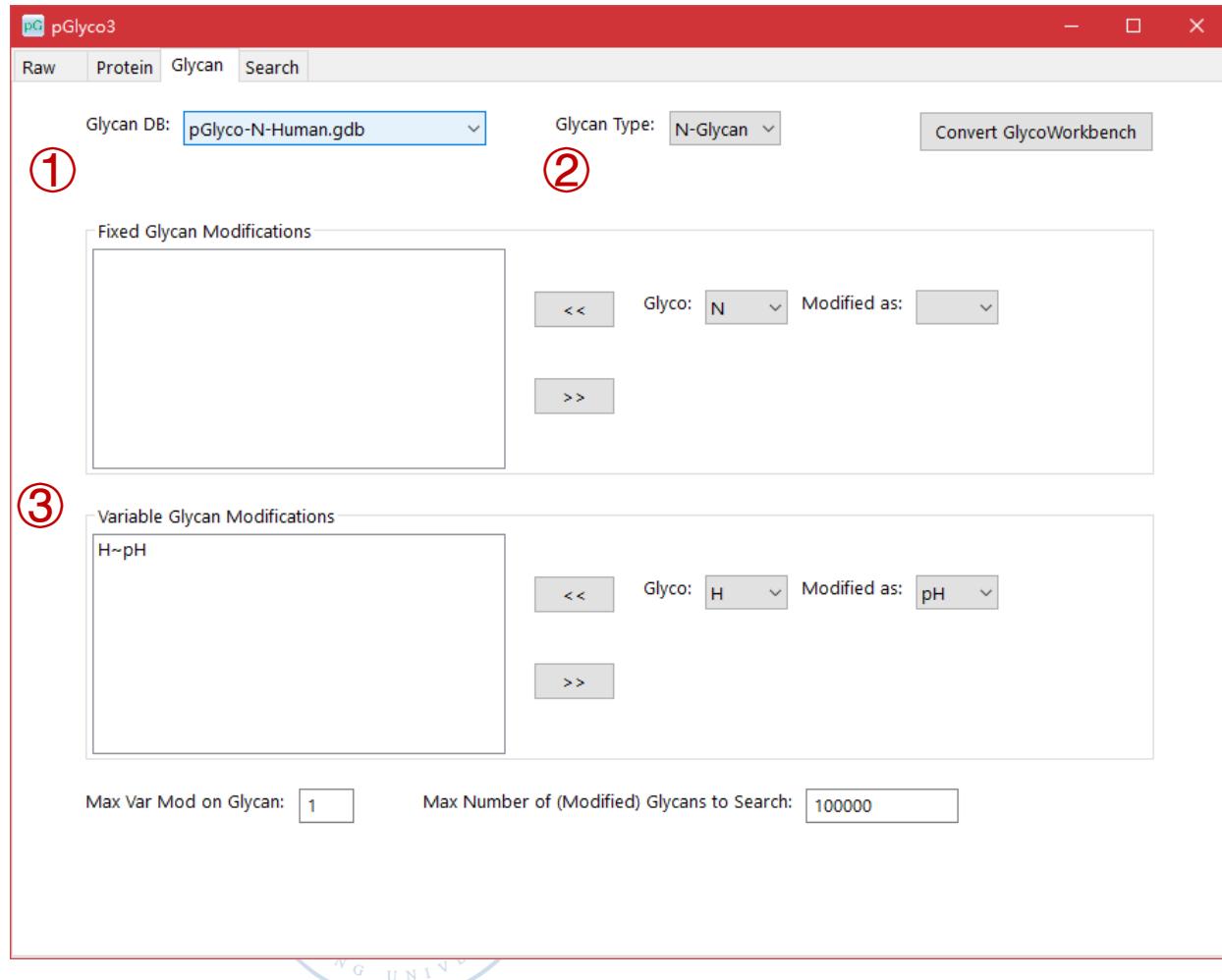
TMT



- ① Set the fasta database.
- ② Set the trypsin enzyme.
- ③ Set the modification information like the left panel.
- ④ Set the filter information.

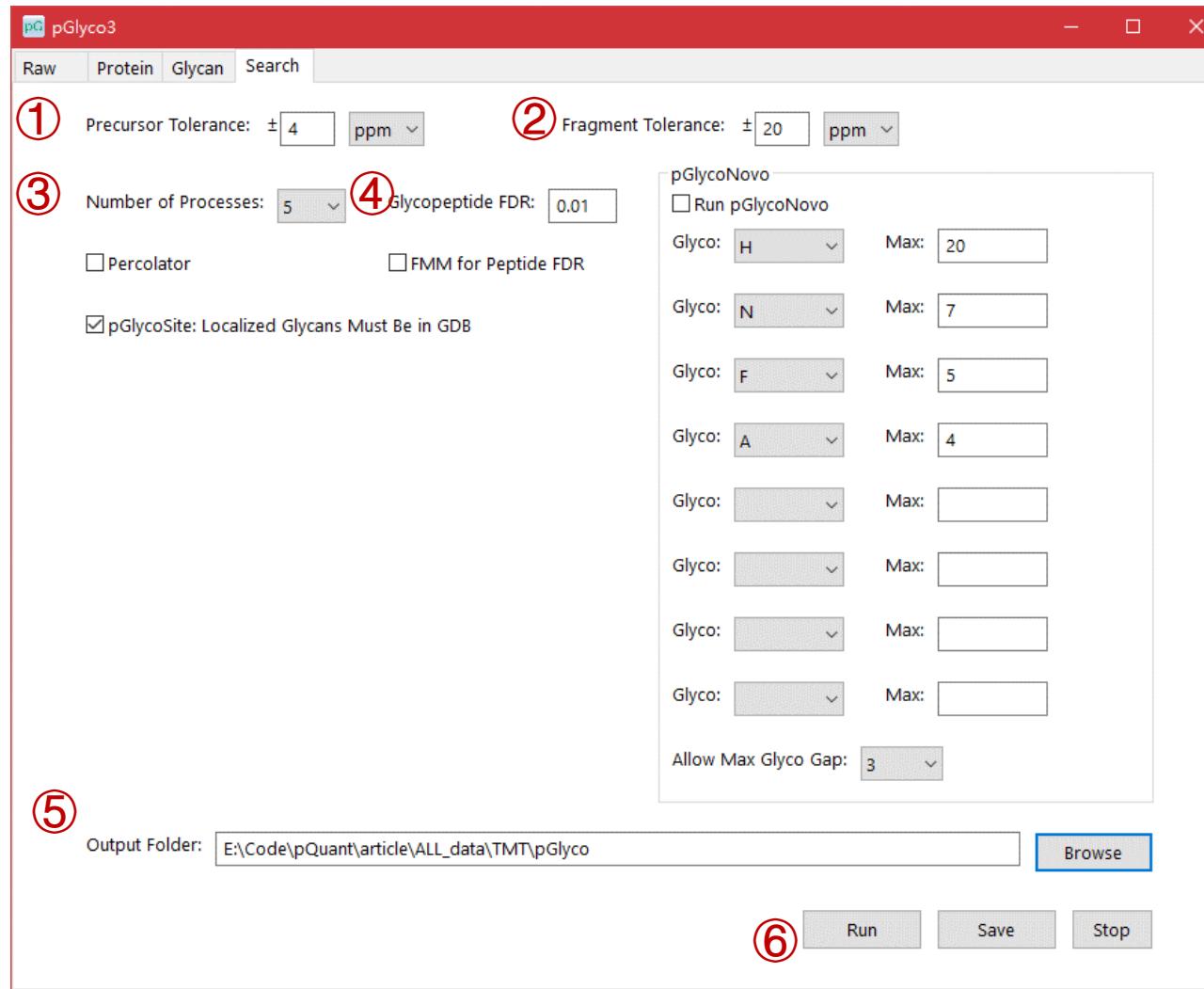
1 Identification with pGlyco

TMT



- ① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.
- ② Set the **Glycan Type** as **N-Glycan**.
- ③ Set the Glycan modification information.

1 Identification with pGlyco



- 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>pGlyc3GUI.exe  
Already registered!  
E:\Code\pQuant\article\ALL_data\SILAC\pGlyco.cfg  
E:\Code\pQuant\article\ALL_data\SILAC\pGlyco.cfg  
E:\Code\pQuant\article\ALL_data\SILAC\pGlyco.cfg  
[pGlyco] Starting task ...  
Process ID=14610: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\rem *****  
*****  
Process ID=14610: E:\Code\pQuant\article\software\pGlyco\pGlyco3_0_build20210615\bin\parse_raw.exe "E:\Code\pQuant\article\ALL_data\SILAC\pGlyco.cfg"  
Process (pid=38324) has been killed Raw: 2.50%  
Process (pid=14610) has been killed  
Process (pid=10172) has been killed  
Process (pid=14610) has been killed  
E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\pGlyco.cfg  
E:\Code\pQuant\article\ALL_data\SILAC\pGlyco\pGlyco.cfg  
[pGlyco] Starting task ...  
Process ID=14618: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\rem *****  
*****  
Process ID=14618: E:\Code\pQuant\article\software\pGlyco\pGlyco3.0_build20210615\bin\parse_raw.exe "E:\Code\pQuant\article\ALL_data\SILAC\pGlyco.cfg"  
Process ID=14618: [pGlyco] *****  
Process ID=14618: / pParse2.2 (x64) from pfmid Studio \  
Process ID=14618: ( Email : pfmid@ict.ac.cn )  
Process ID=14618: Website: http://pfmid.ict.ac.cn \  
Process ID=14618: *****  
Process ID=14618: The license will expire in 2100-1-1  
Process ID=14618: [pParse] [INFO] pParse writes logs in E:\Code\pQuant\article\ALL_data\SILAC\pParsePlusLog.txt  
Process ID=14618: [pParse] [INFO] ----- BEGIN PARAMETERS -----  
Process ID=14618: [pParse] [INFO] - 01: check activationcenter = 1
```

1 Identification with pGlyco

TMT

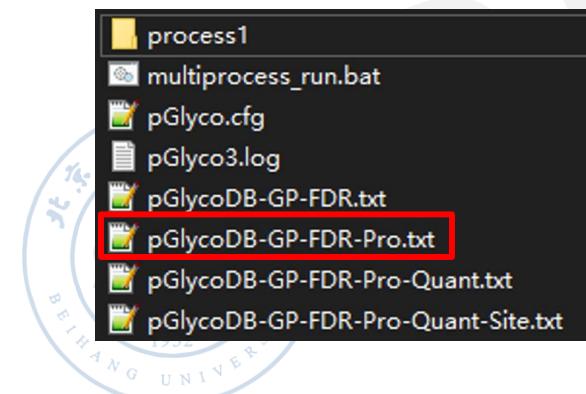
①

```
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>pPercolator.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\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: E:\Code\pQuant\article\ALL_data\TMT\pGlyco\process1\pGlycoDB-GP-Raw1-FDR-noFiltered.txt
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\pQuant\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\article\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.

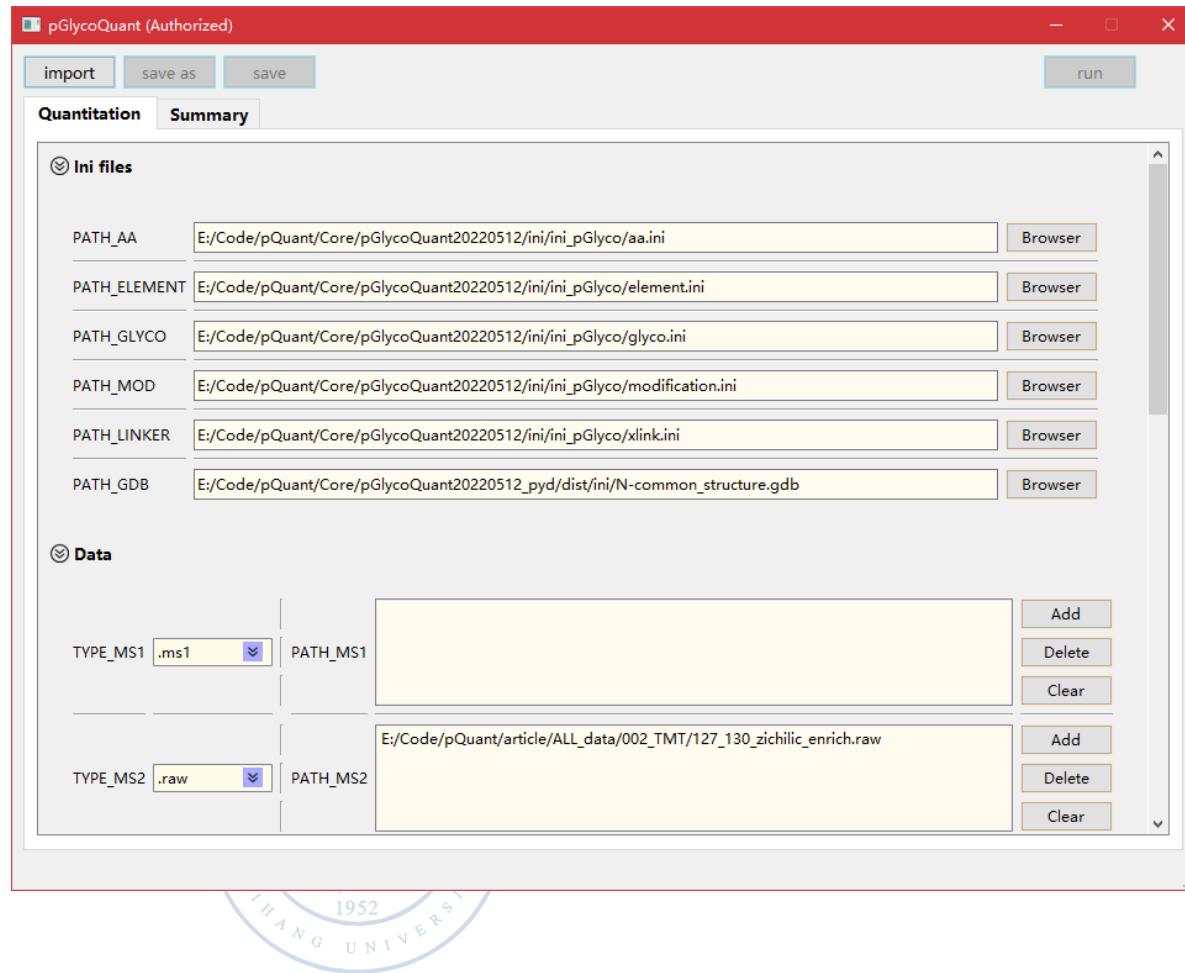
②



2 Quantitation with pGlycoQuant

TMT

2.1 pGlycoQuant quantitation

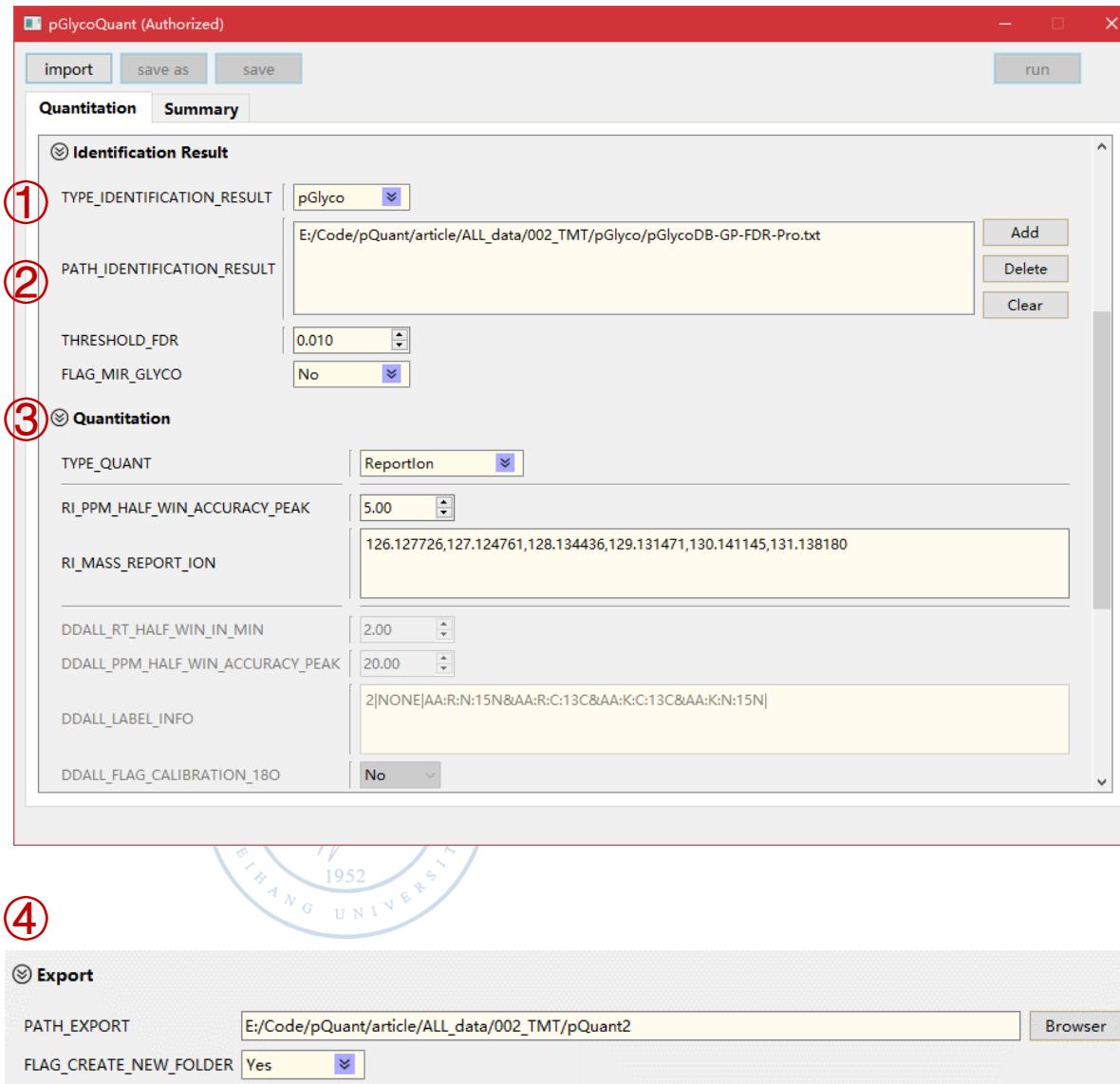


- ① Ensure that the ini file paths are valid.
- ② Set the **TYPE_MS2** as **.raw** and fill the raw files into the **PATH_MS2** blank.

2 Quantitation with pGlycoQuant

TMT

2.1 pGlycoQuant quantitation



- 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_REPORTION** could be

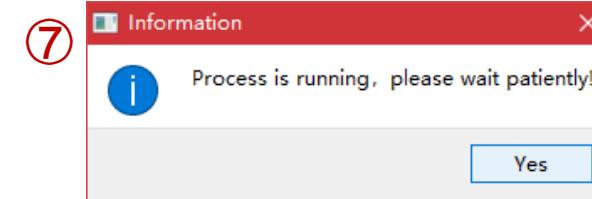
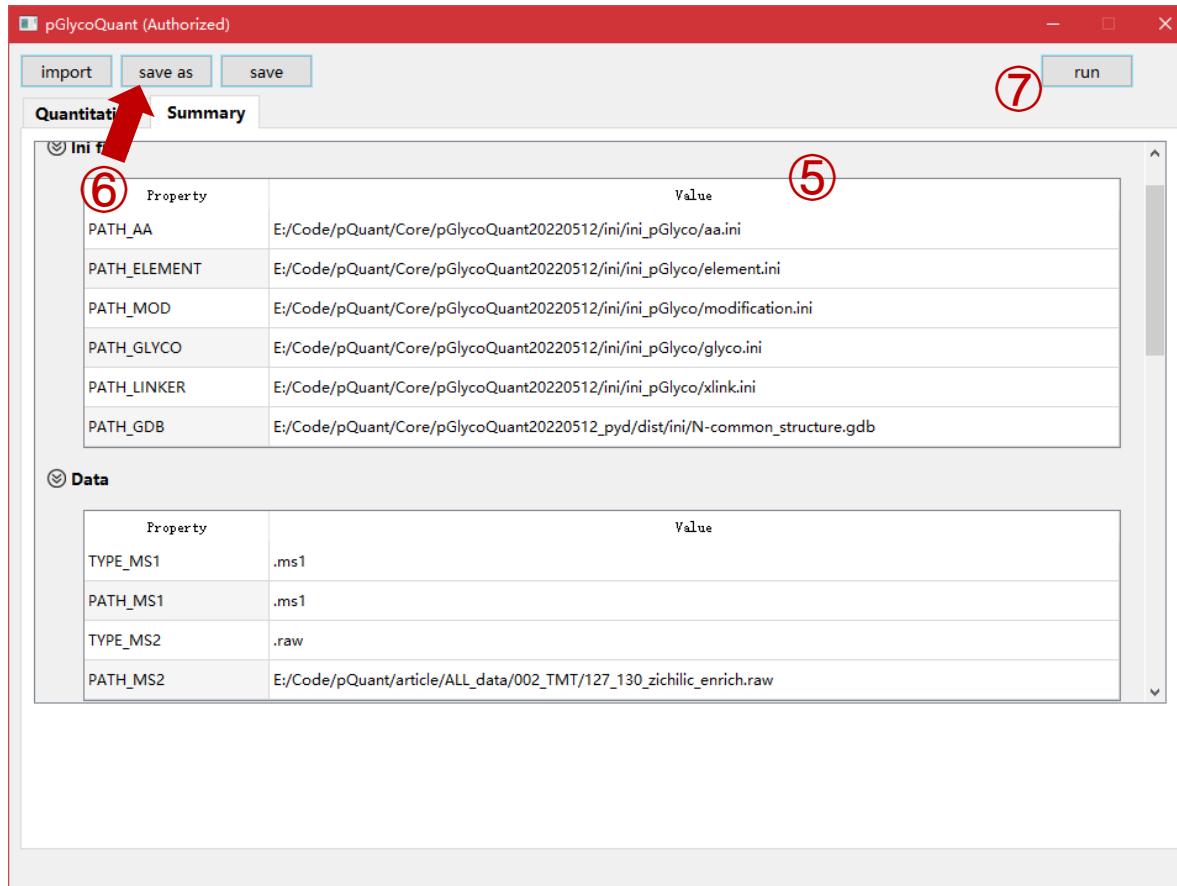
**126.127726,127.124761,128.134436,129.131471,130.141
145,131.138180.**

- ④ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

TMT

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.

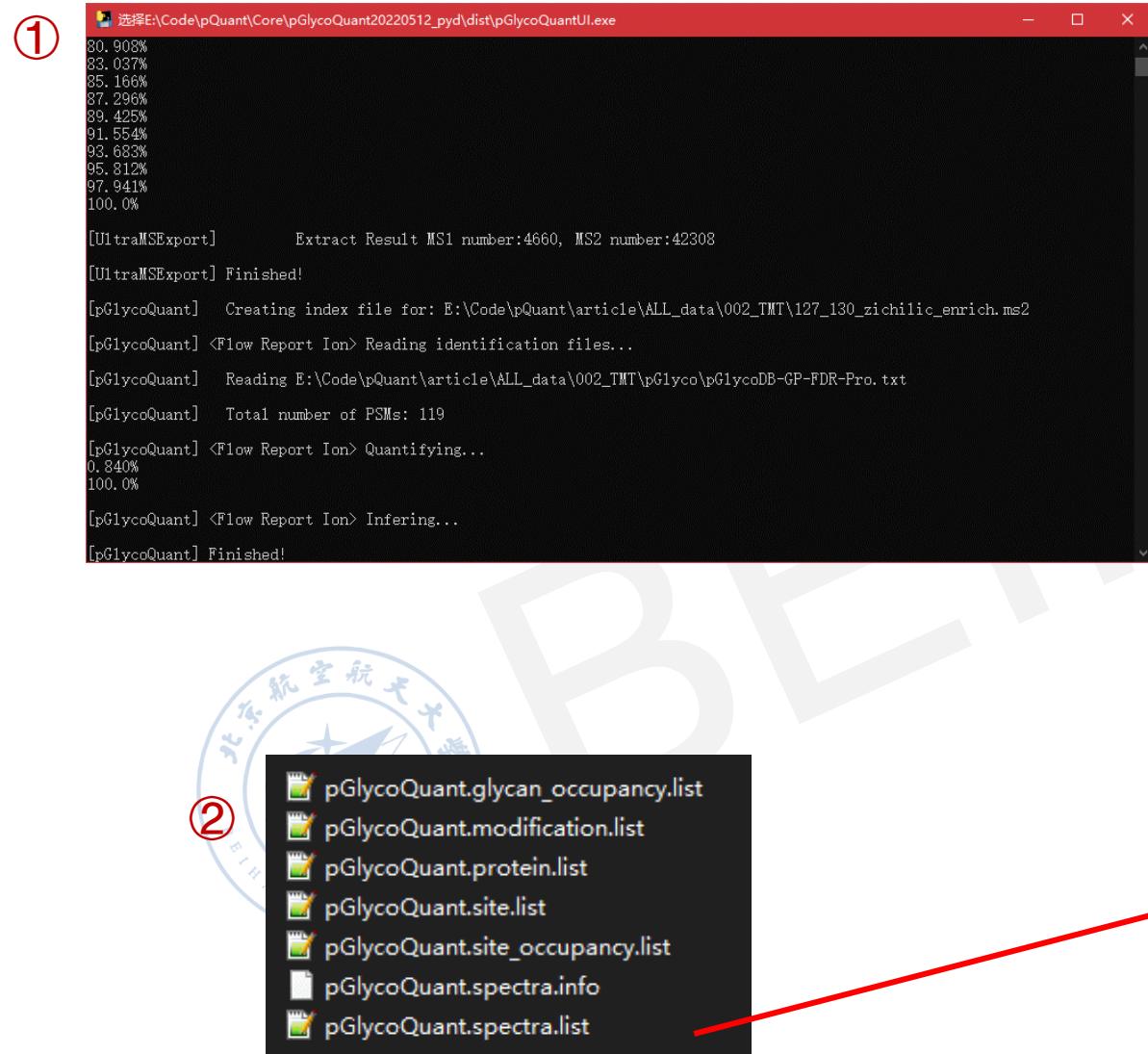
A screenshot of a command-line interface window titled 'E:\Code\pQuant\Core\pGlycoQuant20220512_pyd\dist\pGlycoQuantUI.exe'. The window displays log messages from the pGlycoQuant process. It starts with copyright information for Beihang University, followed by environment checks, ini file reading, and MS2 file reading. The 'UltraMSExport' component is mentioned for extracting a RAW file. Below this, a long list of percentage values is displayed, ranging from 0.002% to 34.068%, likely representing the progress of the quantitation process. A red circle with the number 7 is placed over the top-left corner of the window.

```
[pGlycoQuant] Copyright 2021 Beihang University. All rights reserved. Version 2022.05  
2022-05-15 12:48:55.596255  
[pGlycoQuant] <Flow Report Ion> Checking the environment...  
[pGlycoQuant] <Flow Report Ion> Reading ini files...  
[pGlycoQuant] <Flow Report Ion> Reading MS2 files (The first run will take a few minutes)...  
[UltraMSExport] Copyright 2020 Beihang University. All rights reserved. Version 2020.07  
[UltraMSExport] Extract RAW File: E:\Code\pQuant\article\ALL_data\002_TMT\127_130_zichilic_enrich.raw  
0.002%  
2.131%  
4.260%  
6.389%  
8.519%  
10.648%  
12.777%  
14.906%  
17.035%  
19.164%  
21.293%  
23.422%  
25.551%  
27.681%  
29.810%  
31.939%  
34.068%
```

2 Quantitation with pGlycoQuant

TMT

2.2 pGlycoQuant results



- 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				
GlySpec	PepSpec	RawName	Scan	RT	Precursor	Precursor	Charge	Rank	Peptide	Mod	Peptide	M-Glycan	N-Glycan	Con	Plausible	Glyc				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17				
2	127_130_z127_130_z127_130_z1	8693	2044.456	2732.231	1366.619	2	1	JLEEK	1TMT6ple	1191.699	7.2	0	0	H(7)N(2)	(NN)NH(H)-	11421	0	1	0	
3	127_130_z127_130_z127_130_z1	16470	3826.016	3785.689	1262.568	3	1	LGTDPIVA1TMT6ple		1759	10	2	0	0	H(7)N(2)	(NN)NH(H)-	10340	0	1	0
4	127_130_z127_130_z127_130_z1	18386	4266.49	4318.653	1080.469	4	1	HNDTQH1TMT6ple		2940.371	6	2	0	0	H(6)N(2)	(NN)NH(H)-	11463	0	1	0
5	127_130_z127_130_z127_130_z1	18436	4278.043	4156.8	1039.955	4	1	HNDTQH1TMT6ple		2940.371	5	2	0	0	H(5)N(2)	(NN)NH(H)-	11518	0	1	0
6	127_130_z127_130_z127_130_z1	14934	3473.993	3173.375	1058.463	3	1	FNYQQTIV1TMT6ple		1470.79	8	2	0	0	H(8)N(2)	(NN)NH(H)-	11452	0	1	0
7	127_130_z127_130_z127_130_z1	3762	902.888	2928.189	1464.598	2	1	GVISACAR1TMT6ple		1063.552	9	2	0	0	H(9)N(2)	(NN)NH(H)-	11358	0	1	0
8	127_130_z127_130_z127_130_z1	32315	7454.437	4185.739	1395.918	3	1	VMSWWD1TMT6ple		2321.104	9	2	0	0	H(9)N(2)	(NN)NH(H)-	14673	0	1	0
9	127_130_z127_130_z127_130_z1	10858	2546.11	3106.41	1036.142	3	1	DAVNJITA1TMT6ple		1403.826	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10345	0	1	0
10	127_130_z127_130_z127_130_z1	8700	2046.138	2732.23	911.415	3	1	JLTEEK	1TMT6ple	1191.699	7	2	0	0	H(7)N(2)	(NN)NH(H)-	10935	0	1	0
11	127_130_z127_130_z127_130_z1	6832	1614.429	2666.108	1333.558	2	1	NLMSR	1TMT6ple	963.5243	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10352	0	1	0
12	127_130_z127_130_z127_130_z1	17869	4148.071	3516.618	1758.813	2	1	VFGSQLT1TMT6ple		1651.978	9	2	0	0	H(9)N(2)	(NN)NH(H)-	10930	0	1	0
13	127_130_z127_130_z127_130_z1	23445	5414.001	4660.085	1165.777	4	1	TDEQEVQ1TMT6ple		2967.501	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10258	0	1	0
14	127_130_z127_130_z127_130_z1	9928	2332.427	2496.17	832.7281	3	1	SUCLTVK1TMT6ple		1279.744	5	2	0	0	H(5)N(2)	(NN)NH(H)-	14652	0	1	0
15	127_130_z127_130_z127_130_z1	26316	6074.413	4163.808	1041.708	4	1	VOLEDFE1TMT6ple		2087.07	6	4	0	0	H(6)N(2)P(G)O(N)H(H)-	(NN)NH(H)-	11551	0	1	0
16	127_130_z127_130_z127_130_z1	11846	2773.553	3150.329	1575.668	2	1	JYTADYK1TMT6ple		1447.747	8	2	0	0	H(8)N(2)	(NN)NH(H)-	11505	0	1	0
17	127_130_z127_130_z127_130_z1	4857	1157.516	3564.518	1188.844	3	1	YHSQTYG1TMT6ple		1699.69	9	2	0	0	H(9)N(2)	(NN)NH(H)-	10342	0	1	0
18	127_130_z127_130_z127_130_z1	8934	2099.288	2944.359	982.1246	3	1	JATLAEQA1TMT6ple		1403.826	7	2	0	0	H(7)N(2)	(NN)NH(H)-	11421	0	1	0
19	127_130_z127_130_z127_130_z1	9595	2254.935	2962.329	994.7811	3	1	SLCTVK1TMT6ple		1279.744	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10258	0	1	0
20	127_130_z127_130_z127_130_z1	15997	3717.018	3300.414	1100.809	3	1	JTSWDNA1TMT6ple		1597.837	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10352	0	1	0
21	127_130_z127_130_z127_130_z1	18289	4244.351	4642.952	1161.494	4	1	HNDTQH1TMT6ple		2940.371	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10352	0	1	0
22	127_130_z127_130_z127_130_z1	17753	4121.404	3140.457	1047.491	3	1	VVYLTKE1TMT6ple		1437.872	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10352	0	1	0
23	127_130_z127_130_z127_130_z1	19971	4628.824	4257.981	1065.251	4	1	TTLDINT1TMT6ple		2555.398	8	2	0	0	H(8)N(2)	(NN)NH(H)-	10345	0	1	0
24	127_130_z127_130_z127_130_z1	8789	2068.808	3268.465	1090.16	3	1	JATLAEQA1TMT6ple		1403.826	9	2	0	0	H(9)N(2)	(NN)NH(H)-	10946	0	1	0
25	127_130_z127_130_z127_130_z1	20062	4650.036	3183.49	1061.835	3	1	IVPLUK1TMT6ple		1318.85	9	2	0	0	H(9)N(2)	(NN)NH(H)-	10930	0	1	0
26	127_130_z127_130_z127_130_z1	24531	5664.887	3703.717	926.6847	4	1	IFIQQTGIE1TMT6ple		1839.078	9	2	0	0	H(9)N(2)	(NN)NH(H)-	9762	0	1	0
27	127_130_z127_130_z127_130_z1	30710	7087.175	4897.149	1225.043	4	1	IIISPEEQTV1TMT6ple		2659.381	7	4	1	0	H(7)N(4)A	(NN)NH(H)-	11074	0	1	0
28	127_130_z127_130_z127_130_z1	14847	3453.878	3335.429	1112.481	3	1	FINYQQTIV1TMT6ple		1470.79	9	2	0	0	H(9)N(2)	(NN)NH(H)-	10342	0	1	0
29	127_130_z127_130_z127_130_z1	22649	5233.488	4307.966	1077.747	4	1	AITTQPGN1TMT6ple		2070.196	7	4	1	0	H(7)N(4)A	(NN)NH(H)-	11074	0	1	0
30	127_130_z127_130_z127_130_z1	20451	4739.856	3689.682	923.1759	4	1	LGTDPIVA1TMT6ple		1759	6	4	0	0	H(6)N(2)P(G)O(N)H(H)-	(NN)NH(H)-	11560	0	1	0
31	127_130_z127_130_z127_130_z1	26291	6068.664	4163.907	1388.607	3	1	VDLEDFE1TMT6ple		2087.07	6	4	0	0	H(6)N(4)F(G)O(N)H(H)-	(NN)NH(H)-	2376	0	1	0
32	127_130_z127_130_z127_130_z1	7845	1846.799	3354.642	1188.896	3	1	GPGBKJQ1TMT6ple		1814.11	7	2	0	0	H(7)N(2)	(NN)NH(H)-	11421	0	1	0
33	127_130_z127_130_z127_130_z1	18102	4310.407	3502.606	1200.207	2	1	FLCJASD1TMT6ple		1722.060	9	2	0	0	H(8)N(2)	(NN)NH(H)-	10227	0	1	0

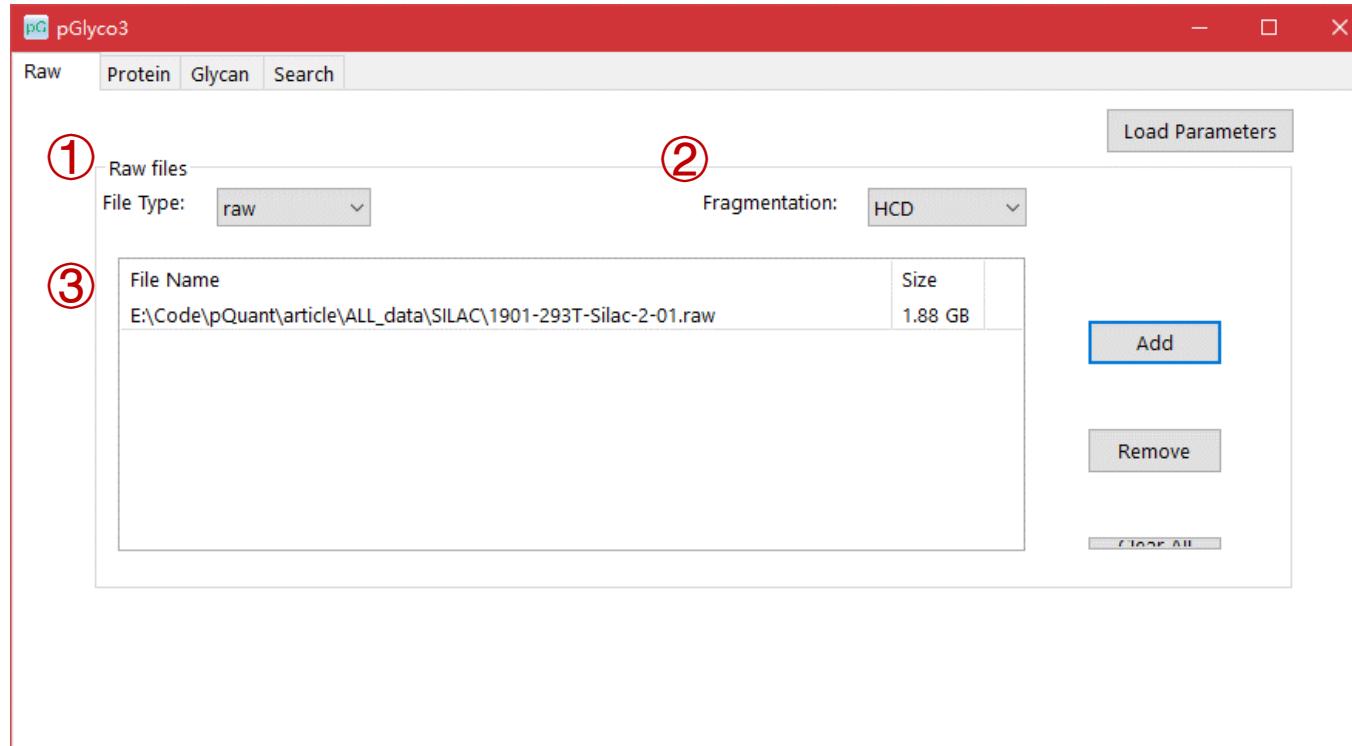
pGlyco & pGlycoQuant

Manual for SILAC Data



1 Identification with pGlyco

SILAC

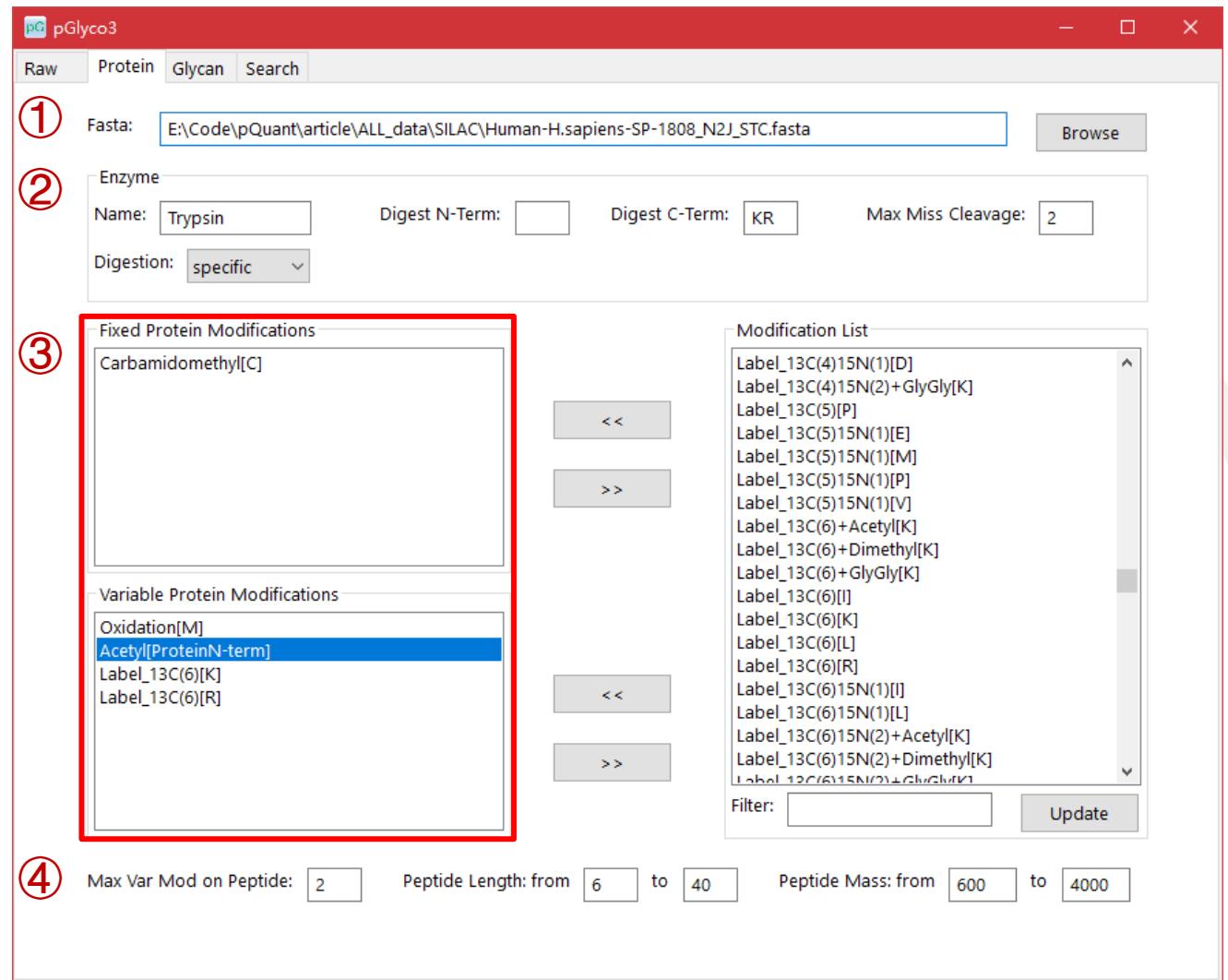


- ① Load raw files.
- ② Set the **Fragmentation** as **HCD**.
- ③ Add the **SILAC** raw file.



1 Identification with pGlyco

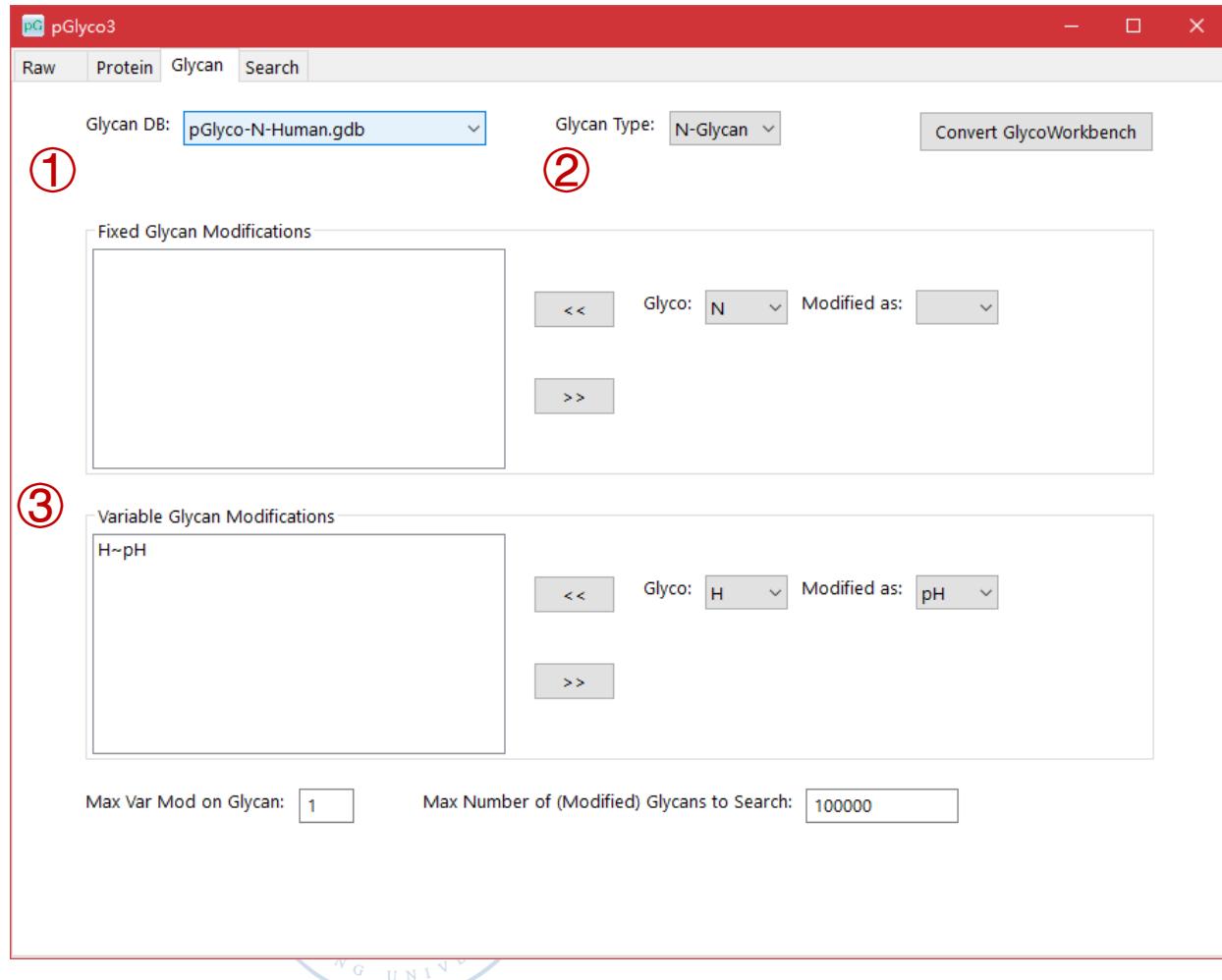
SILAC



- ① Set the fasta database.
- 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

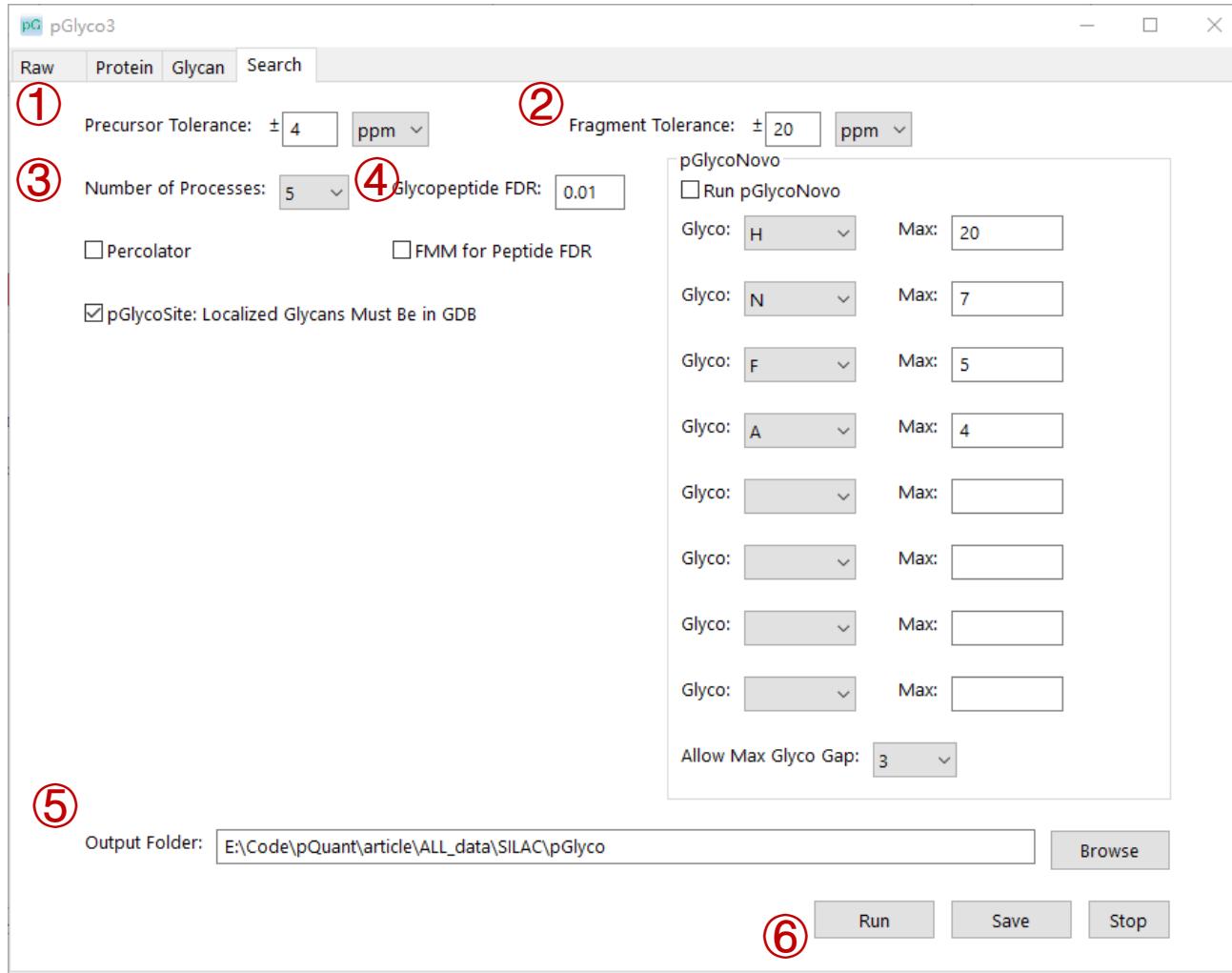
SILAC



- ① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.
- ② Set the **Glycan Type** as **N-Glycan**.
- ③ Set the Glycan modification information.

1 Identification with pGlyco

SILAC



- 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.

A command-line window titled "C:\WINDOWS\system32\cmd.exe" is shown. The user has navigated to the bin directory of the pGlyco3 software and run the "parse_raw.exe" command with the "-rem" option. The command-line output shows the software's configuration and the start of the processing. A red arrow points from the "Run" button in the pGlyco3 interface to this command-line window.

```
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
[pGlyco] 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_data\SILAC\pGlyco\process1\pGlyco.cfg"
Process ID=21796: [pGlyc *****]
Process ID=21796: / pParse2.2 (x64) from pFind Studio
Process ID=21796: < Email: pfind@ict.ac.cn
Process ID=21796: Website: www.pfind.ict.ac.cn
Process ID=21796: *****
Process ID=21796: The license will expire in 2100-1-1
Process ID=21796: [pParse] (INFO) - pParse writes logs in E:\Code\pQuant\article\All_data\SILAC\pParsePlusLog.txt
Process ID=21796: [pParse] (INFO) - ----- BEGIN PARAMETERS -----
Process ID=21796: [pParse] (INFO) - 01: check_activationcenter = 1
Process ID=21796: [pParse] (INFO) - 02: correlation = 1
Process ID=21796: [pParse] (INFO) - 03: cut_similar_mono = 1
Process ID=21796: [pParse] (INFO) - 04: datarun = 1
Process ID=21796: [pParse] (INFO) - 05: datapath = E:\Code\pQuant\article\All_data\SILAC\1901-293T-Silac-2-01.msl
Process ID=21796: [pParse] (INFO) - 06: delete_msn = 0
Process ID=21796: [pParse] (INFO) - 07: dia_mode = 0
Process ID=21796: [pParse] (INFO) - 08: dia_mode_to_filter_by_selectedMS2scanNoSet = 1
Process ID=21796: [pParse] (INFO) - 09: input_format = msl
Process ID=21796: [pParse] (INFO) - 10: intensity = 1
```

1 Identification with pGlyco

SILAC

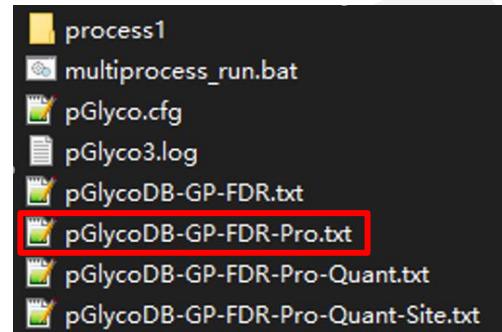
①

```
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.pf1
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.

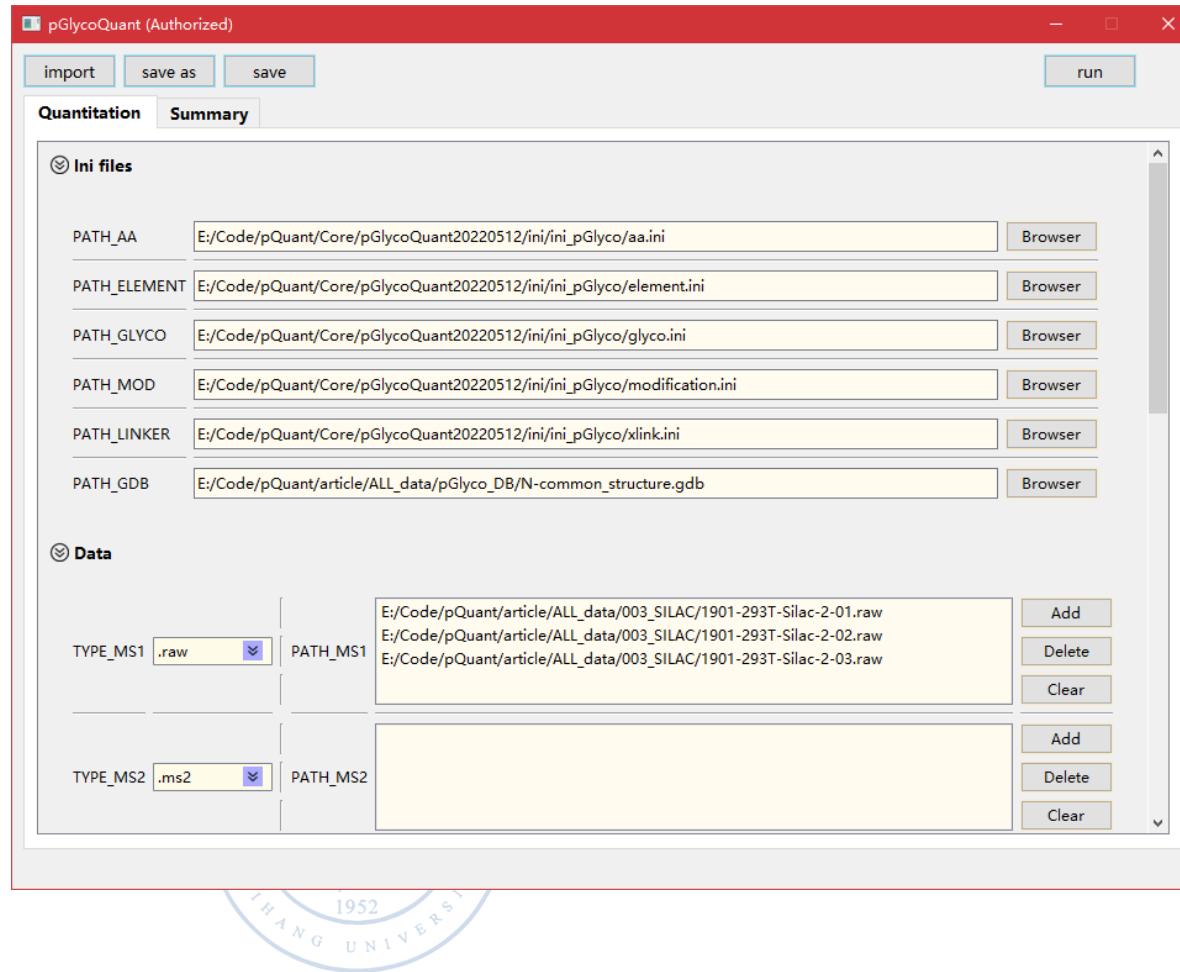
②



2 Quantitation with pGlycoQuant

SILAC

2.1 pGlycoQuant quantitation

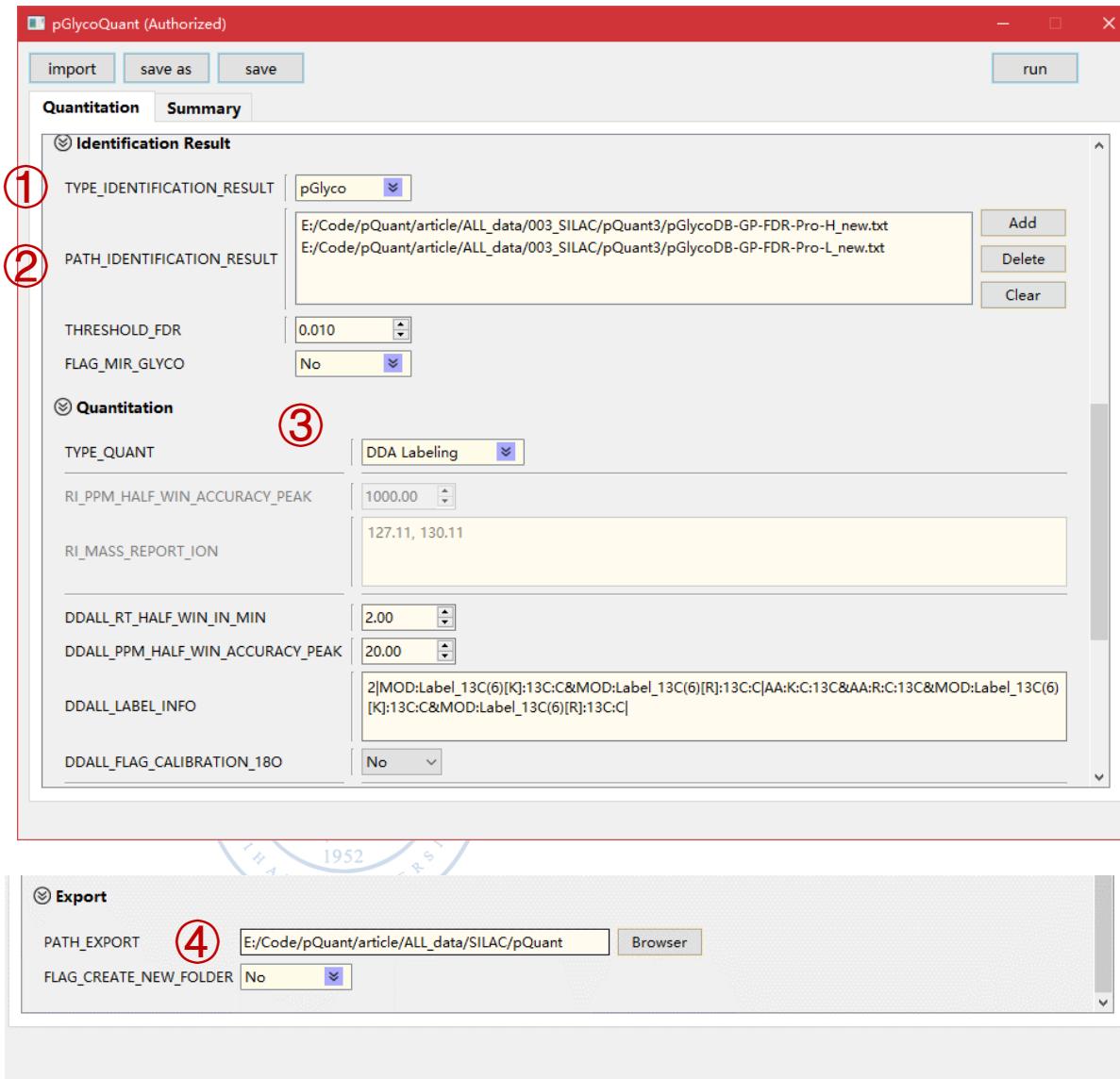


- ① Ensure that the ini file paths are valid.
- ② Set the **TYPE_MS1** as **.raw** and fill the raw files into the **PATH_MS1** blank.

2 Quantitation with pGlycoQuant

SILAC

2.1 pGlycoQuant quantitation

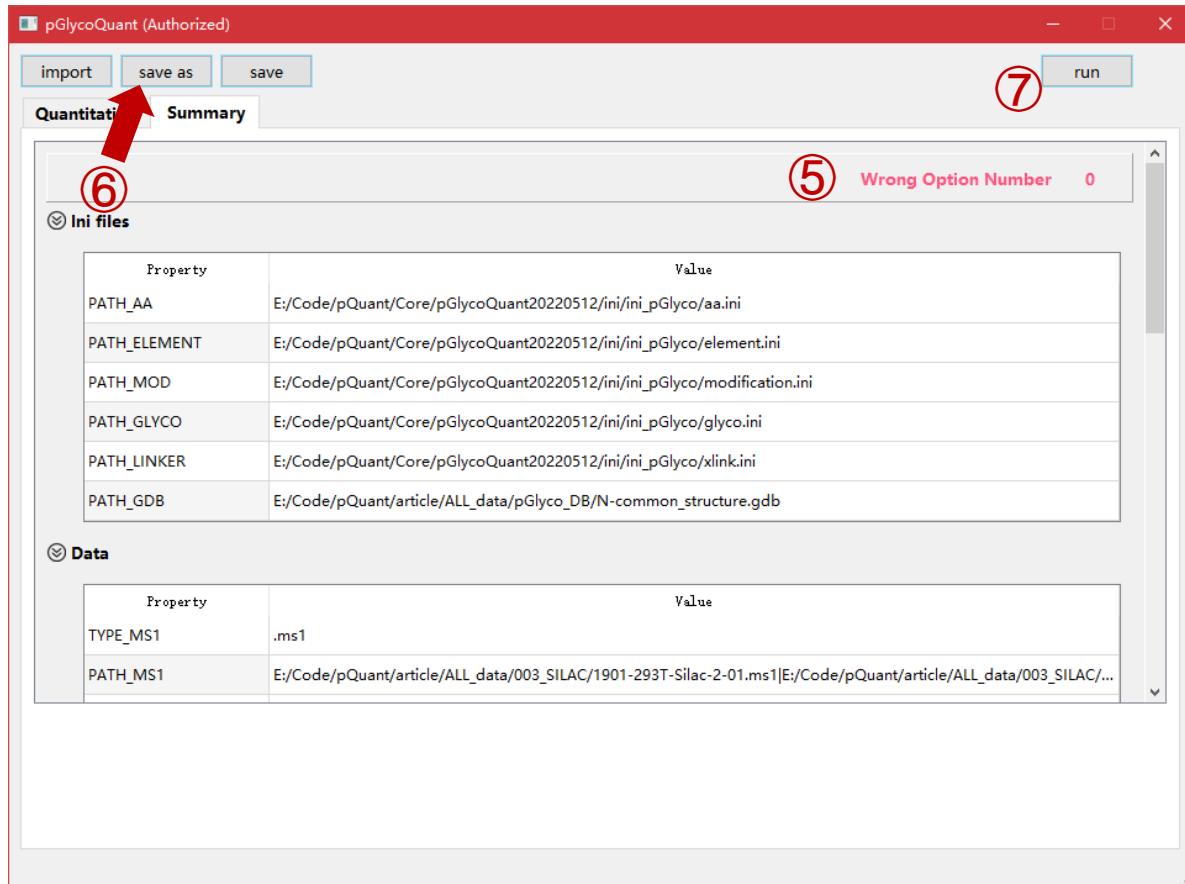


- ① Set **TYPE_IDENTIFICATION_RESULT** as **pGlyco**.
- ② Put identification results in **PATH_IDENTIFICATION_RESULT** blank and set **FDR** as **0.01**.
- ③ Set **TYPE_QUANT** as **DDA Labeling**.
Set **DDALL_RT_HALF_WIN_IN_MIN** as **2.00** minutes (default) and set **DDALL_PPM_HALF_WIN_ACCURACY_PEAK** 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.

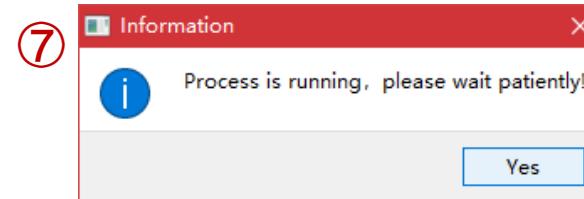
2 Quantitation with pGlycoQuant

SILAC

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.



A command-line interface window titled '选择E:\Code\pQuant\Core\pGlycoQuant20220512\pyd\dist\pGlycoQuantUI.exe' is shown. The window displays log messages from the pGlycoQuant process. The text includes:

```
[pGlycoQuant] Copyright 2021 Beihang University. All rights reserved. Version 2022.05  
2022-05-15 21:20:08.960+27  
[pGlycoQuant] <Flow Labeling> Checking the environment...  
[pGlycoQuant] <Flow Labeling> Reading ini files...  
[pGlycoQuant] <Flow Labeling> Reading identification files...  
[pGlycoQuant] Reading E:\Code\pQuant\article\ALL_data\003_SILAC\pQuant3\pGlycoDB-GP-FDR-Pro-H_new.txt  
[pGlycoQuant] Total number of PSMs: 2197  
[pGlycoQuant] Reading E:\Code\pQuant\article\ALL_data\003_SILAC\pQuant3\pGlycoDB-GP-FDR-Pro-L_new.txt  
[pGlycoQuant] Total number of PSMs: 4492  
[pGlycoQuant] <Flow Labeling> Reading MS1 files (The first run will take a few minutes)...  
[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%  
13.39%  
17.85%  
22.31%  
26.78%  
31.19%  
35.64%
```

2 Quantitation with pGlycoQuant

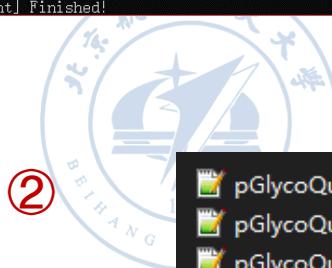
SILAC

2.2 pGlycoQuant results

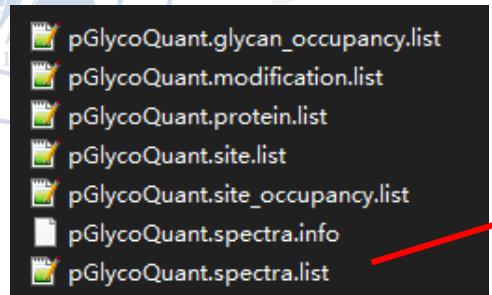
①

```
E:\Code\pQuant\Core\pGlycoQuant20220512_pyd\dist\pGlycoQuantUI.exe

[pGlycoQuant] Creating index file for: E:\Code\pQuant\article\ALL_data\003_SILAC\1901-293T-Silac-2-01.msl
[pGlycoQuant] Creating index file for: E:\Code\pQuant\article\ALL_data\003_SILAC\1901-293T-Silac-2-02.msl
[pGlycoQuant] Creating index file for: E:\Code\pQuant\article\ALL_data\003_SILAC\1901-293T-Silac-2-03.msl
[pGlycoQuant] <Flow Labeling> Quantifying...
0.0%
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



① The completed information.

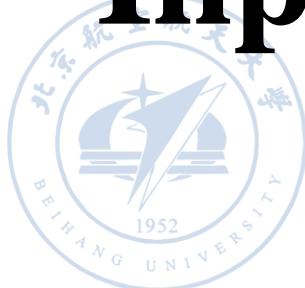
② The quantitation results. Please open the files with Excel.

#	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	GlySpec	PepSpec	RawName	Scan	RT	Precursor	PrecursorChARGE	Rank	Peptide	Mod	PeptideM/Glycan(H,N)GlycanConPlausibleStGlyID	GlyFrag					
1																	
2	1901-293T1901-293T1901-293T	37314	1007455	2779288	927.1007			3	TJSTFVQA14Label_1		1578.8584 2 0 1 0	H4(N)2(F1(N)F1(N)H-	29 0 1 0 0 0				
3	1901-293T1901-293T1901-293T	19060	5153299	2477076	1239.042			2	JIGQVLEK	8.Label_13	936.5456 7 2 0 0 0	H7(N)2	(NN)NH(H)	114 0 1 0 0 0			
4	1901-293T1901-293T1901-293T	31946	8629107	2409123	1205.065			2	INNVITIQT11Label_1		1192.6995 5 2 0 0 0	H5(N)2	(NN)NH(H)	31 0 1 0 0 0			
5	1901-293T1901-293T1901-293T	3029	8191295	2381977	1191.492			2	SLSISTAR	8.Label_13	841.4477 7 2 0 0 0	H7(N)2	(NN)NH(H)	114 0 1 0 0 0			
6	1901-293T1901-293T1901-293T	47502	1281546	3288455	1096.823			3	LLHALGGC16Label_1		1747.9267 2 2 0 0 0	H7(N)2	(NN)NH(H)	114 0 1 0 0 0			
7	1901-293T1901-293T1901-293T	9991	2702409	3173.289	794.0776			4	YHYGTFE11Label_1		1470.7058 2 0 0 0	H8(N)2	(NN)NH(H)	210 0 1 0 0 0			
8	1901-293T1901-293T1901-293T	9773	2643.202	2860.0777	1430.542			2	JYTADVK	8.Label_13	995.4412 9 2 0 0 0	H9(N)2	(NN)NH(H)	344 0 1 0 0 0			
9	1901-293T1901-293T1901-293T	24506	6621231	2912.326	971.4468			3	LEJTTGYT15Label_1		1650.9015 5 2 0 0 0	H5(N)2	(NN)NH(H)	31 0 1 0 0 0			
10	1901-293T1901-293T1901-293T	3142	8491516	2381977	1191.492			2	SLISTAR	8.Label_13	841.4477 7 2 0 0 0	H7(N)2	(NN)NH(H)	121 0 1 0 0 0			
11	1901-293T1901-293T1901-293T	17344	4689178	3112.367	1038.127			3	WTGHIVT11Label_1		1302.7014 5 0 1 0	H4(N)5(F1(N)F1(N)H-	291 0 1 0 0 0				
12	1901-293T1901-293T1901-293T	16686	4511.996	2908.226	970.08			3	HTNMLTC10Label_1		1205.64 8 2 0 0 0	H8(N)2	(NN)NH(H)	201 0 1 0 0 0			
13	1901-293T1901-293T1901-293T	7274	1968.482	2492.051	1246.529			2	JATLAEQA9Label_1		951.52017 2 0 0 0	H7(N)2	(NN)NH(H)	114 0 1 0 0 0			
14	1901-293T1901-293T1901-293T	29531	7976.149	2683.162	1342.084			2	TCDWLPK2Carbam		1806.8372 2 0 1 0	H2(N)2(F1(N)F1(N)H-	8 0 1 0 0 0				
15	1901-293T1901-293T1901-293T	25042	6863.698	2699.153	900.3893			3	TCDWLPK2Carbam		1822.8322 2 0 1 0	H2(N)2(F1(N)F1(N)H-	8 0 1 0 0 0				
16	1901-293T1901-293T1901-293T	24518	6624.43	2699.155	900.3898			3	TCDWLPK2Carbam		1822.8322 2 0 1 0	H2(N)2(F1(N)F1(N)H-	8 0 1 0 0 0				
17	1901-293T1901-293T1901-293T	15093	4080.771	2250.057	750.6905			3	LKPFLFK	2.Label_13	871.5803 6 2 0 0 0	H6(N)2	(NN)NH(H)	61 0 1 0 0 0			
18	1901-293T1901-293T1901-293T	20275	5478.06	2657.177	1329.092			2	ALSPJSTIS13Label_1		1278.76 2 0 0 0	H6(N)2	(NN)NH(H)	60 0 1 0 0 0			
19	1901-293T1901-293T1901-293T	32846	8871.816	2808.224	1404.616			2	AGPIGTLF14Label_1		1429.7426 2 0 0 0	H6(N)2	(NN)NH(H)	60 0 1 0 0 0			
20	1901-293T1901-293T1901-293T	27224	7355.377	2941.278	981.0974			3	JHSIFLAD13Label_1		1562.8026 2 0 0 0	H6(N)2	(NN)NH(H)	61 0 1 0 0 0			
21	1901-293T1901-293T1901-293T	25658	6932.579	3016.586	754.902			4	TAAAQVG19Label_1		2610.4252 0 2 0 0 0	(N)	(N)	2 0 1 0 0 0			
22	1901-293T1901-293T1901-293T	14954	4042.744	2523.057	1262.032			2	JITSWK	6.Label_13	754.4189 5 4 0 1 0	H5(N)4(F1(N)N(H)-	256 0 1 0 0 0				
23	1901-293T1901-293T1901-293T	31060	8390.148	4213.963	1054.246			4	AKGPKSAA3Label_13		2835.4896 2 0 0 0	H6(N)2	(NN)NH(H)	60 0 1 0 0 0			
24	1901-293T1901-293T1901-293T	3088	834.6995	1799.753	900.3803			2	YETTKJ	6.Label_13	761.3773 2 3 0 1 0	H3(N)2(F1(N)F1(N)H-	14 0 1 0 0 0				
25	1901-293T1901-293T1901-293T	40354	10891.39	3312.499	828.8802			4	TJSTFVQA14Label_1		274.1223 2 3 0 1 0	H3(N)2(F1(N)F1(N)H-	15 0 1 0 0 0				
26	1901-293T1901-293T1901-293T	13643	3689.379	2598.061	1299.534			2	LITCESSK	5.Carbam	1057.5297 2 2 0 0 0	H7(N)2	(NN)NH(H)	114 0 1 0 0 0			
27	1901-293T1901-293T1901-293T	12126	3277.629	2654.102	1327.555			2	DAVNJITA9Label_13		951.52017 2 0 0 0	H8(N)2	(NN)NH(H)	203 0 1 0 0 0			
28	1901-293T1901-293T1901-293T	22204	5996.889	2950.231	1475.619			2	FINYQTVS10Label_1		1247.6478 2 0 0 0	H8(N)2	(NN)NH(H)	215 0 1 0 0 0			
29	1901-293T1901-293T1901-293T	31138	8411.065	2649.111	883.7086			3	DUETIHWV11Label_1		1432.6875 2 0 0 0	H5(N)2	(NN)NH(H)	31 0 1 0 0 0			
30	1901-293T1901-293T1901-293T	9970	2696.716	2664.093	1332.55			2	GPTJTTCV17Carbam		1123.5627 2 0 0 0	H7(N)2	(NN)NH(H)	114 0 1 0 0 0			
31	1901-293T1901-293T1901-293T	19506	5272.749	2209.938	1105.473			2	YVETEMDV10Label_1		1155.5566 4 2 0 0 0	H4(N)2	(NN)NH(H)	17 0 1 0 0 0			
32	1901-293T1901-293T1901-293T	13061	3522.085	2591.131	864.3817			3	NHTASILE10Label_1		1146.5963 4 3 0 1 0	H3(N)4(F1(N)F1(N)H-	87 0 1 0 0 0				
33	1901-293T1901-293T1901-293T	12640	2417.62	1042.700	872.200			2	TCETMMWV17Label_12		1020.1924 2 2 0 0 0	H4(N)2	(NN)NH(H)	10 0 1 0 0 0			

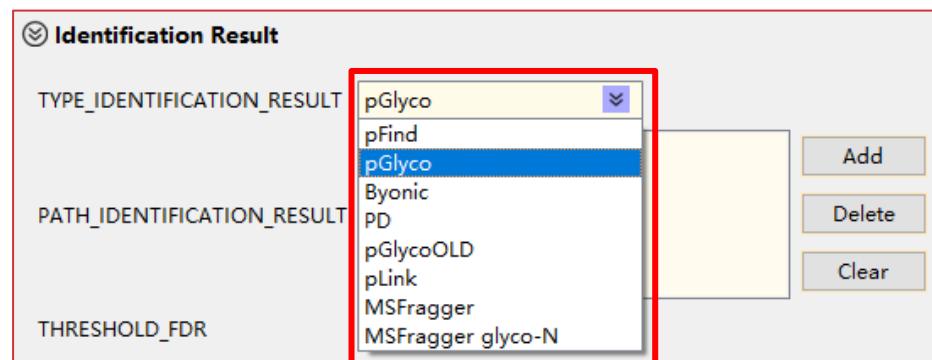
BEIHANG

Notes for Choosing the

Input File for pGlycoQuant

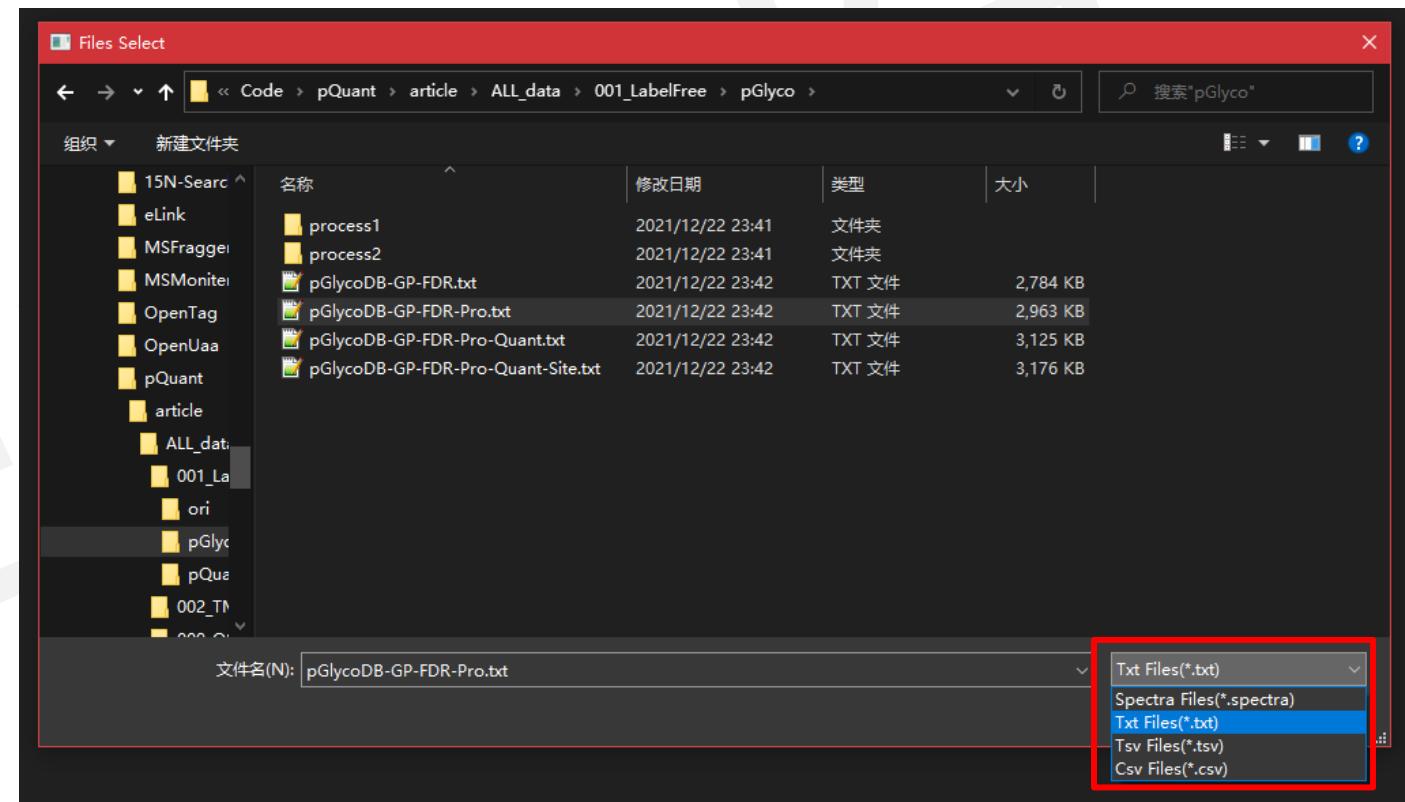


Choose the related type of identification result for quantitation

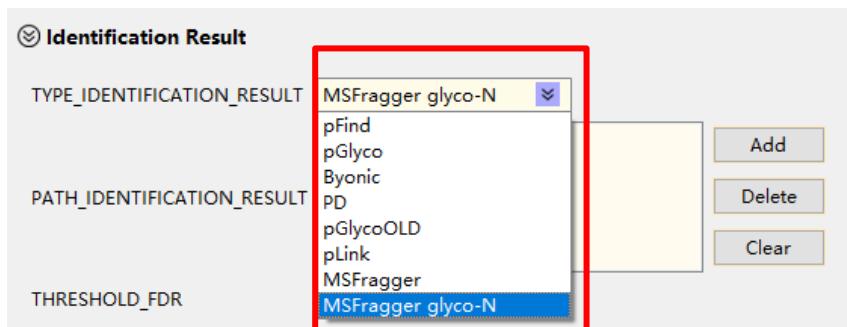


pGlyco results

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

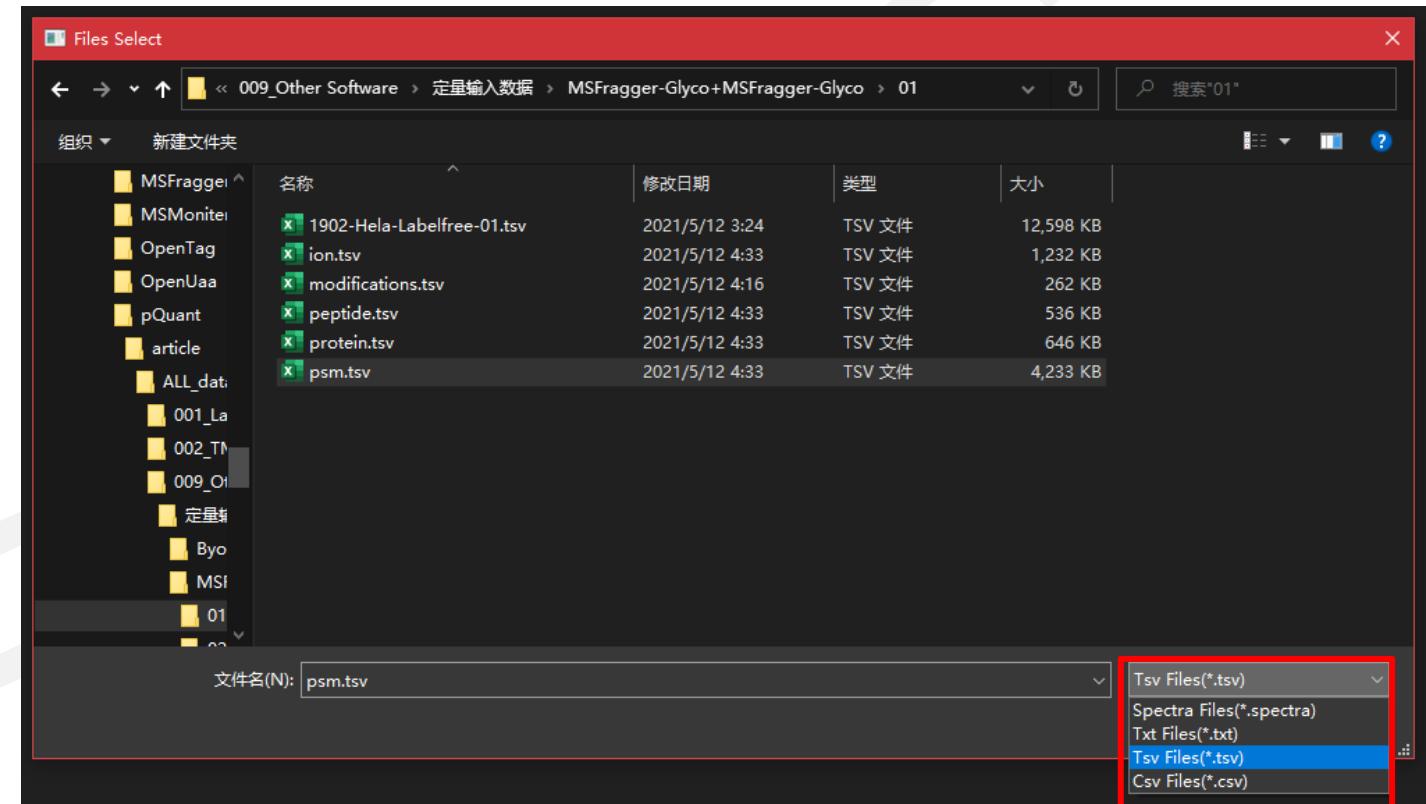


Choose the related type of identification result for quantitation

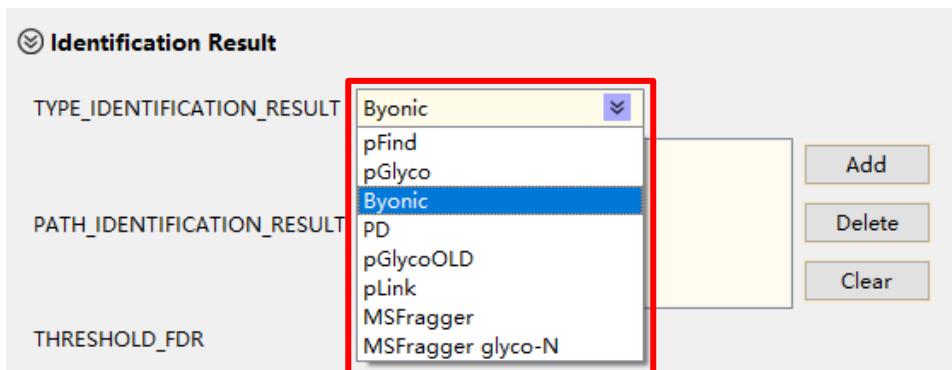


MSFragger results

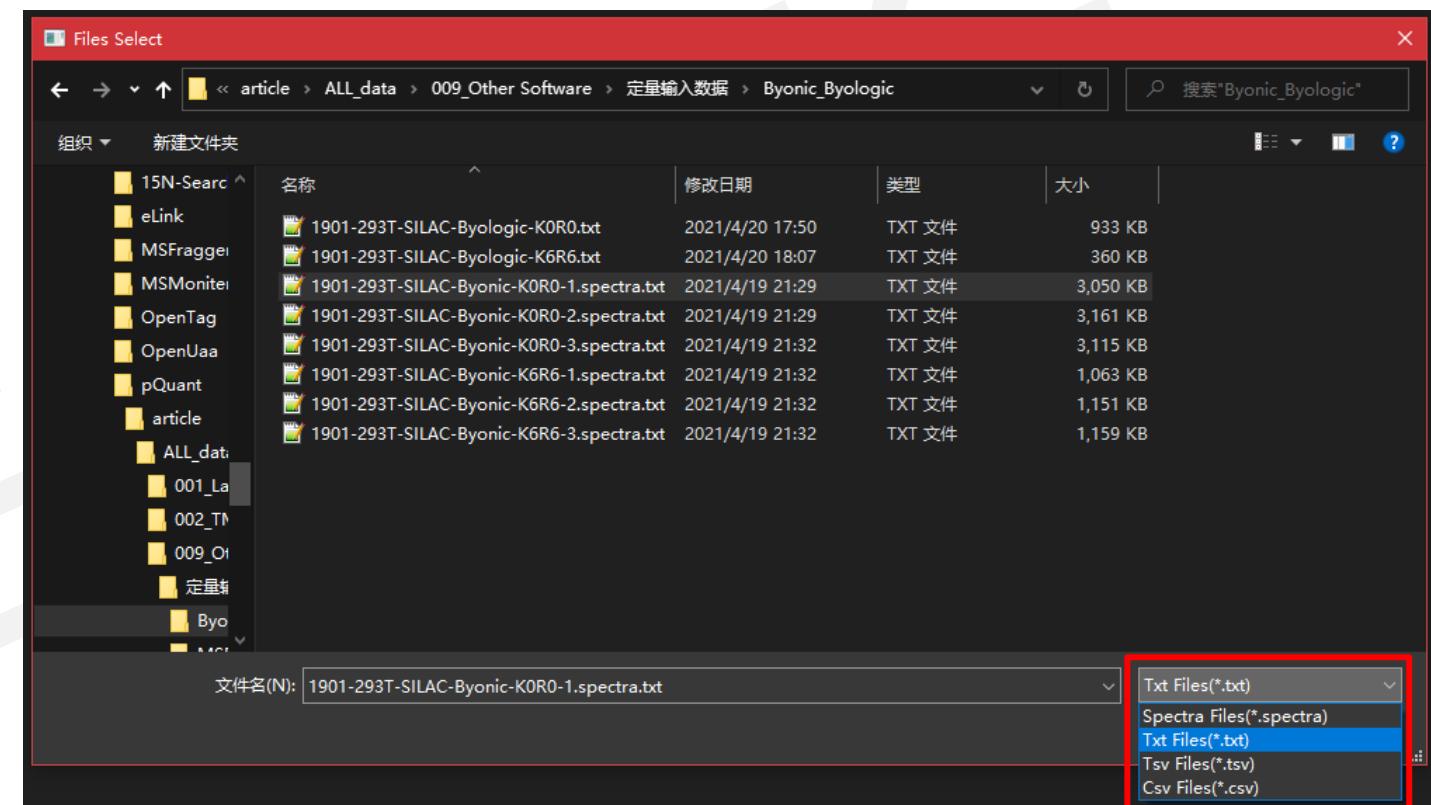
1902-Hela-Labelfree-01.pepXML	50,753 KB
1902-Hela-Labelfree-01.tsv	12,598 KB
1902-Hela-Labelfree-01_model.png	72 KB
1902-Hela-Labelfree-01_quant.csv	10,217 KB
delta-mass.html	300 KB
filter.log	3 KB
interact.pep.xml	34,841 KB
ion.tsv	1,232 KB
modifications.tsv	262 KB
peptide.tsv	536 KB
protein.fas	2,212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



Choose the related type of identification result for quantitation



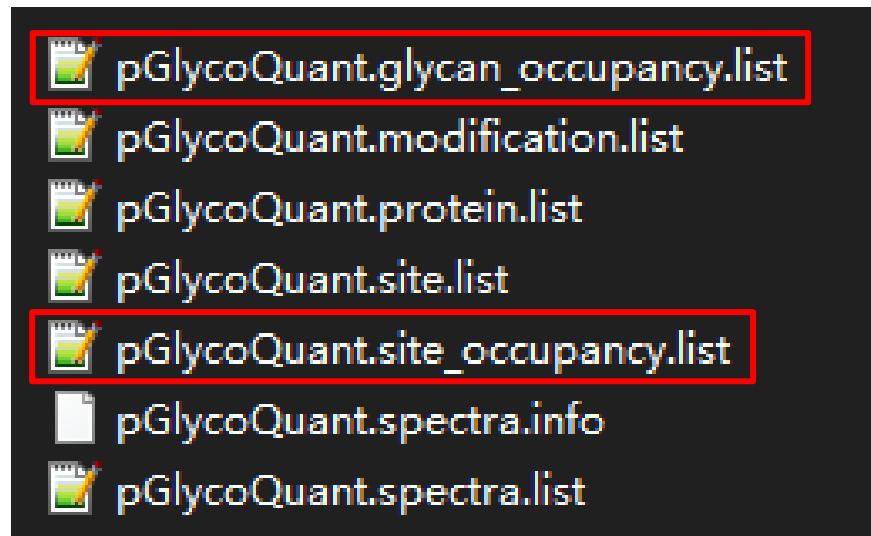
Byonic results			
	1901-293T-SILAC-Byologic-K0R0.txt	933 KB	
	1901-293T-SILAC-Byologic-K0R0.xlsx	406 KB	
	1901-293T-SILAC-Byologic-K6R6.txt	360 KB	
	1901-293T-SILAC-Byologic-K6R6.xlsx	163 KB	
	1901-293T-SILAC-Byonic-K0R0-1.spectra.txt	3,050 KB	
	1901-293T-SILAC-Byonic-K0R0-1.xlsx	1,640 KB	
	1901-293T-SILAC-Byonic-K0R0-2.spectra.txt	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	





The New Function for Glycan Type/Site Statistics

In v202209 version, we added a new function for glycan type/site statistics, “pGlycoQuant.glycan_occupancy.list” and “pGlycoQuant.site_occupancy.list” are listed in the result output folder for your further research.



- ① The “glycan_occupancy.list” gives the quantitative information of different glycan compositions and glycan types at a same glycosylation site.
- ② The “site_occupancy.list” gives the quantitative information of the same glycan composition at different glycosylation sites on a protein.





pGlycoQuant.glycan_occupancy.list



pGlycoQuant.modification.list



pGlycoQuant.protein.list



pGlycoQuant.site.list



pGlycoQuant.site_occupancy.list



pGlycoQuant.spectra.info



pGlycoQuant.spectra.list

Column C lists all the glycan compositions at a protein glycosylation site, and column D corresponds to the quantitation ratio of each glycan to the total intensity of this glycosylation site. Column E to H shows the quantitation ratio of five glycan types, oligomannose, complex/hybrid, sialylation, fucosylation, and M6P, respectively.

-  pGlycoQuant.glycan_occupancy.list
-  pGlycoQuant.modification.list
-  pGlycoQuant.protein.list
-  pGlycoQuant.site.list
-  pGlycoQuant.site_occupancy.list
-  pGlycoQuant.spectra.info
-  pGlycoQuant.spectra.list

Column C lists all the glycosylation sites of a glycoprotein, which were modified by a certain glycan composition, and column D corresponds to the quantitation ratio of each site that is modified by a certain glycan to the total intensity of this glycoprotein.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Protein	Glycan(H,N,A,F)	Glycan_Specific_Sites	Site_ratio	Empty	1902-HeLa								
2	sp P56199 ITA1_HUMAN	[8,2,0]	460;1113;418;883;840;1102;	3.127e-01;6.375e-02;1.942e-01;3.001e-01;9.996e-02;2.938e-02;		4.7E+10	4.75E+10	4.67E+10	5.53E+10	4.66E+10	4.69E+10	4.8E+10	4.77E+10	4.66E+10
3	sp P32004 L1CAM_HUMAN	[7,2,0]	479;849;979;	2.911e-02;9.700e-01;8.487e-04;		6.05E+09	6.03E+09	5.99E+09	6.34E+09	5.97E+09	5.78E+09	6.11E+09	5.75E+09	5.76E+09
4	sp P11047 LAMC1_HUMAN	[6,2,0]	1107;1241;1395;	1.195e-01;2.060e-01;6.745e-01;		1.45E+09	1.53E+09	1.44E+09	1.53E+09	1.5E+09	1.45E+09	1.39E+09	1.48E+09	1.5E+09
5	sp Q92820 GGH_HUMAN	[6,2,0]	203;	1.000e+00;		7.4E+08	7.62E+08	7.58E+08	1.29E+09	8.96E+08	8.94E+08	9.16E+08	9.23E+08	8.96E+08
6	sp Q96AY3 FKB10_HUMAN	[3,4,0]	407;70;393;	1.745e-01;6.334e-01;1.921e-01;		1.03E+10	1E+10	1.03E+10	1.19E+10	1.03E+10	9.92E+09	1.11E+10	1.1E+10	9.73E+09
7	sp P20645 MPRD_HUMAN	[5,4,1]	83;	1.000e+00;		1.16E+10	1.19E+10	1.17E+10	1.24E+10	1.17E+10	1.17E+10	1.24E+10	1.17E+10	1.13E+10
8	sp Q9UH99 SUN2_HUMAN	[8,2,0]	636;	1.000e+00;		1.85E+08	1.59E+08	1.33E+08	2.27E+08	2E+08	1.76E+08	2.05E+08	1.78E+08	1.77E+08
9	sp P53634 CATC_HUMAN	[6,2,0]	53;	1.000e+00;		4325309	2737640	3553034	3338774	4596164	2771749	4800731	4874569	4507052
10	sp P07602 SAP_HUMAN	[5,2,0]	426;215;332;101;	6.331e-02;2.856e-01;5.482e-01;1.028e-01;		2.08E+09	2.1E+09	1.95E+09	2.46E+09	2.09E+09	2.02E+09	2.1E+09	1.99E+09	1.92E+09
11	sp Q96AY3 FKB10_HUMAN	[5,2,0]	407;70;393;182;310;	6.344e-01;3.418e-01;6.016e-03;8.619e-03;9.210e-03;		1.14E+10	9.95E+09	1.1E+10	1.23E+10	1.11E+10	9.47E+09	1.54E+10	1.07E+10	1.1E+10
12	sp P01033 TIMP1_HUMAN	[7,2,0]	53;	1.000e+00;		16636967	13609563	16391443	17824676	15055770	15378761	12724991	11539246	16014821
13	sp P11279 LAMP1_HUMAN	[4,4,1]	249;84;	9.935e-01;6.516e-03;		2.84E+09	2.85E+09	2.92E+09	3.22E+09	2.75E+09	2.72E+09	2.59E+09	2.56E+09	2.48E+09
14	sp Q8TEM1 PO210_HUMAN	[9,2,0]	337;405;801;1441;44;	1.200e-03;9.640e-01;1.624e-02;9.747e-03;8.816e-03;		2.4E+10	2.11E+10	1.6E+10	2.35E+10	2.17E+10	1.79E+10	2.05E+10	2.54E+10	2.2E+10
15	sp P13987 CD59_HUMAN	[6,4,1]	43;	1.000e+00;		1.51E+09	1.12E+09	1.23E+09	1.35E+09	9.62E+08	1.01E+09	7.22E+08	9.53E+08	8.93E+08
16	sp Q9Y639 NPTN_HUMAN	[7,2,0]	229;197;	9.817e-01;1.827e-02;		5.97E+08	6.51E+08	6E+08	9.63E+08	6.46E+08	6.41E+08	6.56E+08	6.42E+08	6.06E+08
17	sp P14625 ENPL_HUMAN	[8,2,0]	217;62;445;481;	8.876e-01;1.108e-01;7.539e-04;8.012e-04;		5.16E+10	4.71E+10	4.55E+10	4.57E+10	4.31E+10	4E+10	4.6E+10	3.89E+10	3.51E+10
18	sp P07602 SAP_HUMAN	[3,2,0]	215;332;101;426;80;	6.275e-01;7.426e-02;2.711e-01;2.636e-02;8.456e-04;		7.79E+10	7.12E+10	7.75E+10	7.87E+10	7.31E+10	7.3E+10	6.99E+10	6.59E+10	7.25E+10
19	sp O60637 TSN3_HUMAN	[6,2,0]	167;	1.000e+00;		3.68E+09	3.63E+09	3.6E+09	5.45E+09	3.85E+09	3.66E+09	3.78E+09	3.92E+09	3.63E+09
20	sp P13473 LAMP2_HUMAN	[6,2,0]	356;257;	9.969e-01;3.084e-03;		1.42E+10	1.45E+10	1.39E+10	1.53E+10	1.43E+10	1.42E+10	1.5E+10	1.39E+10	1.31E+10

Thanks!



BEIHANG