pGlycoQuant Manual

version.2022.05

Version: pGlycoQuant v202205

Release Date: 2022.05.01

Computer configuration

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

RAM: 16G or higher is recommended

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

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

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

Description

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

Notes for running Byonic result

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

Cite us

Weiqian Cao, et. al. pGlycoQuant with a deep residual network for precise and minuscule-missing-value quantitative glycoproteomics enabling the functional exploration of site-specific glycosylation. bioRxiv 2021.11.15.468561.
doi: https://doi.org/10.1101/2021.11.15.468561

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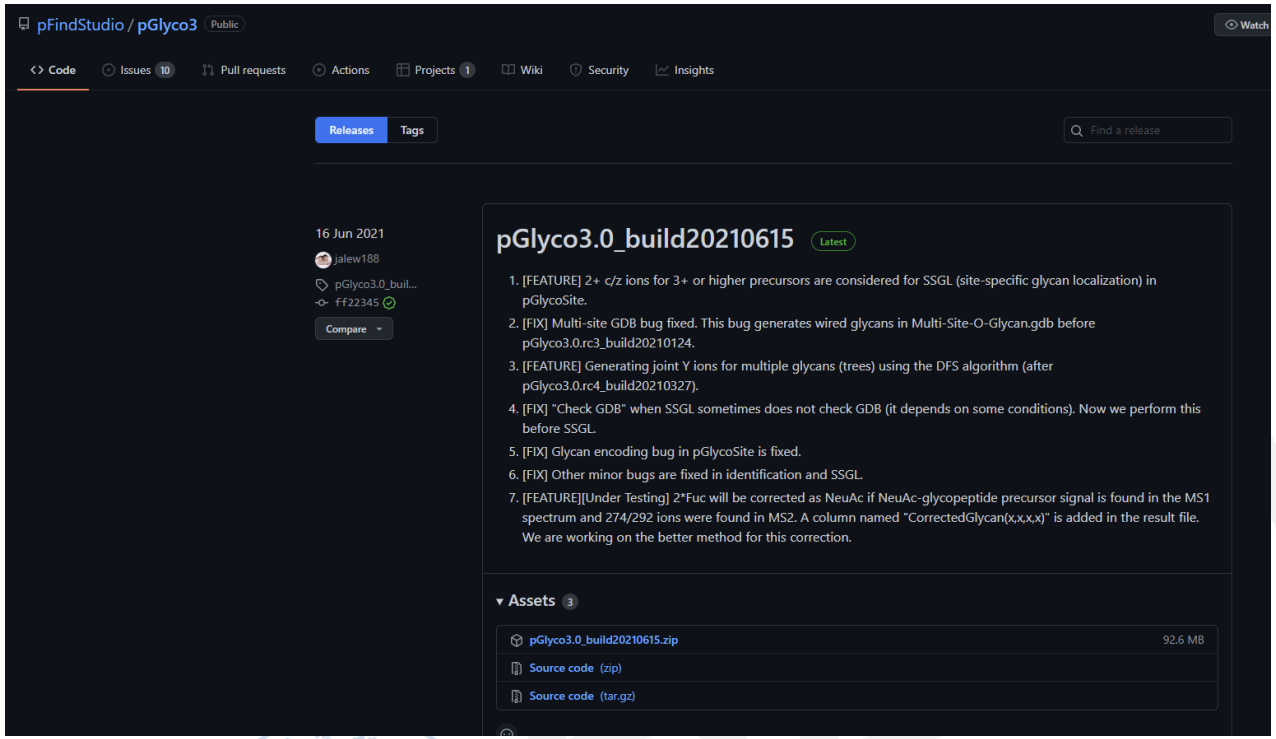
pGlyco & pGlycoQuant

Download and Regist

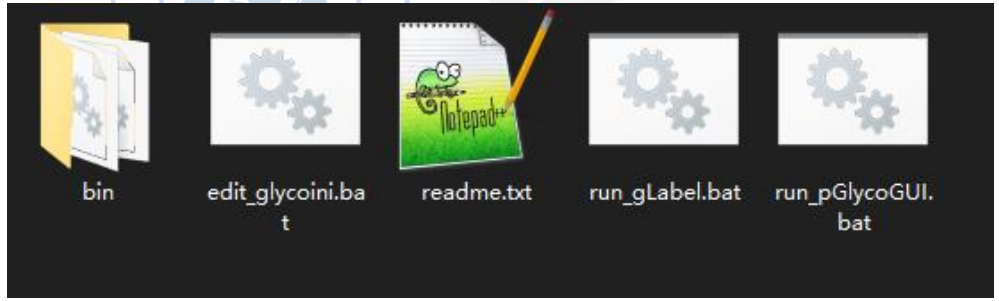
1 Identification with pGlyco

1.1 pGlyco download

①



②

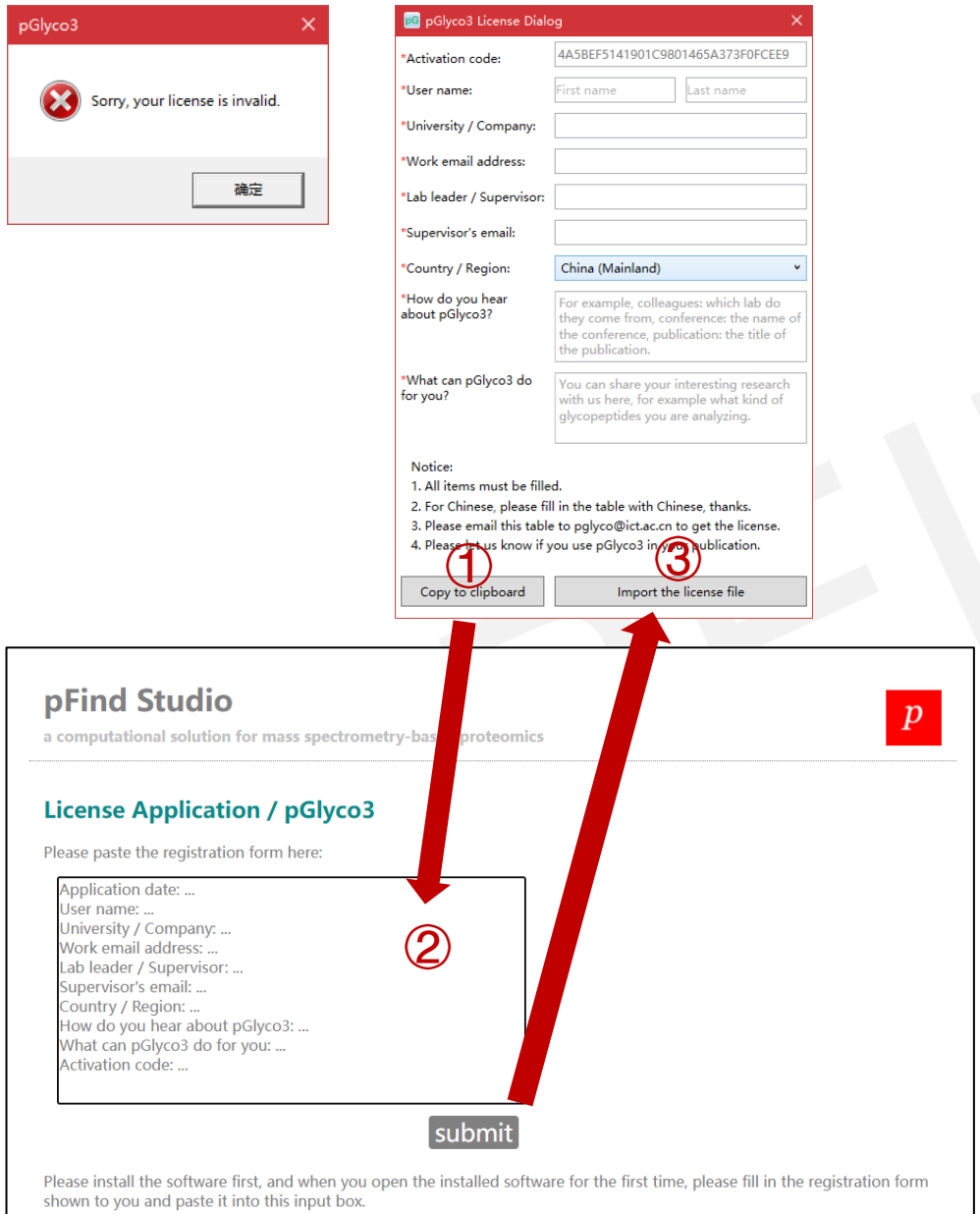


① Login https://github.com/pFindStudio/pGlyco3/releases and download the latest version at the top of the page.

② The unzipped pGlyco files.

1 Identification with pGlyco

1.2 pGlyco register



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

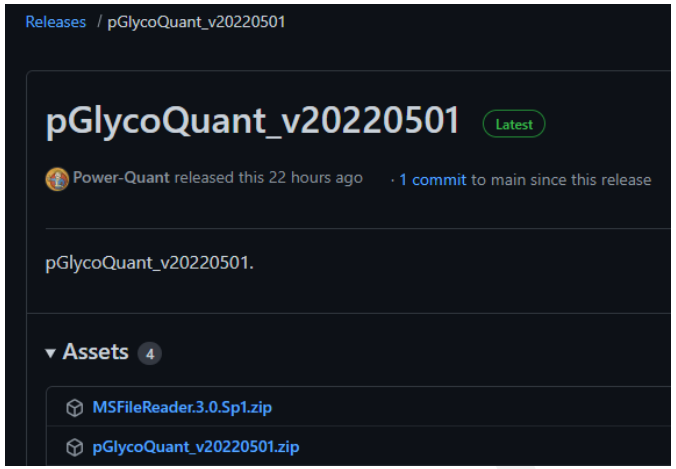
② Login http://i.pfind.org/license/pGlyco3, paste the information and **submit**.

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

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant download

①



②

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

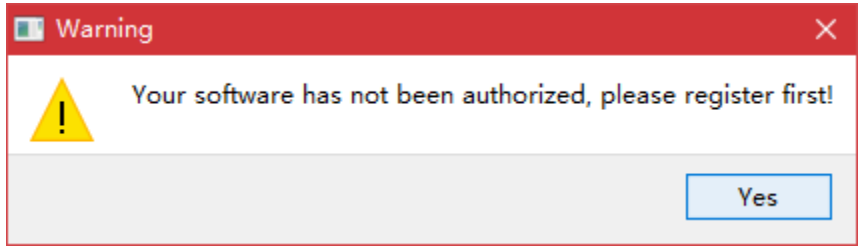
If MSFileReader 3.0 has not been installed, please download **MSFileReader.3.0.Sp1.zip** and install it.

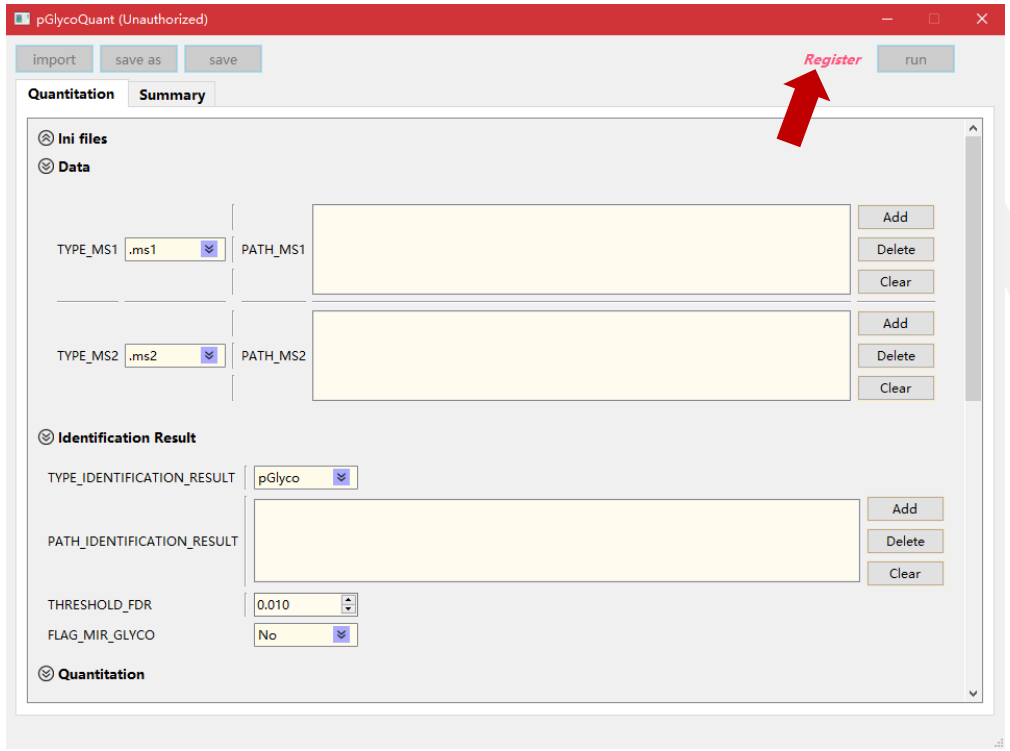
② The unzipped pGlycoQuant files.

2 Quantitation with pGlycoQuant

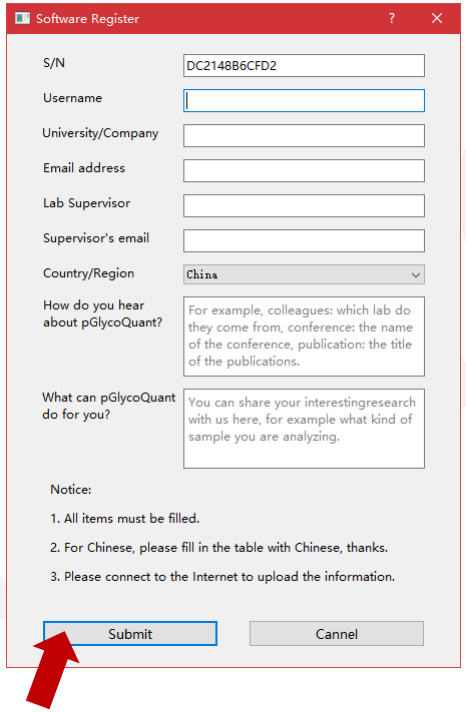
2.2 pGlycoQuant register

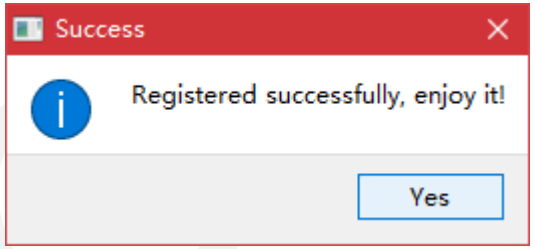
①





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.

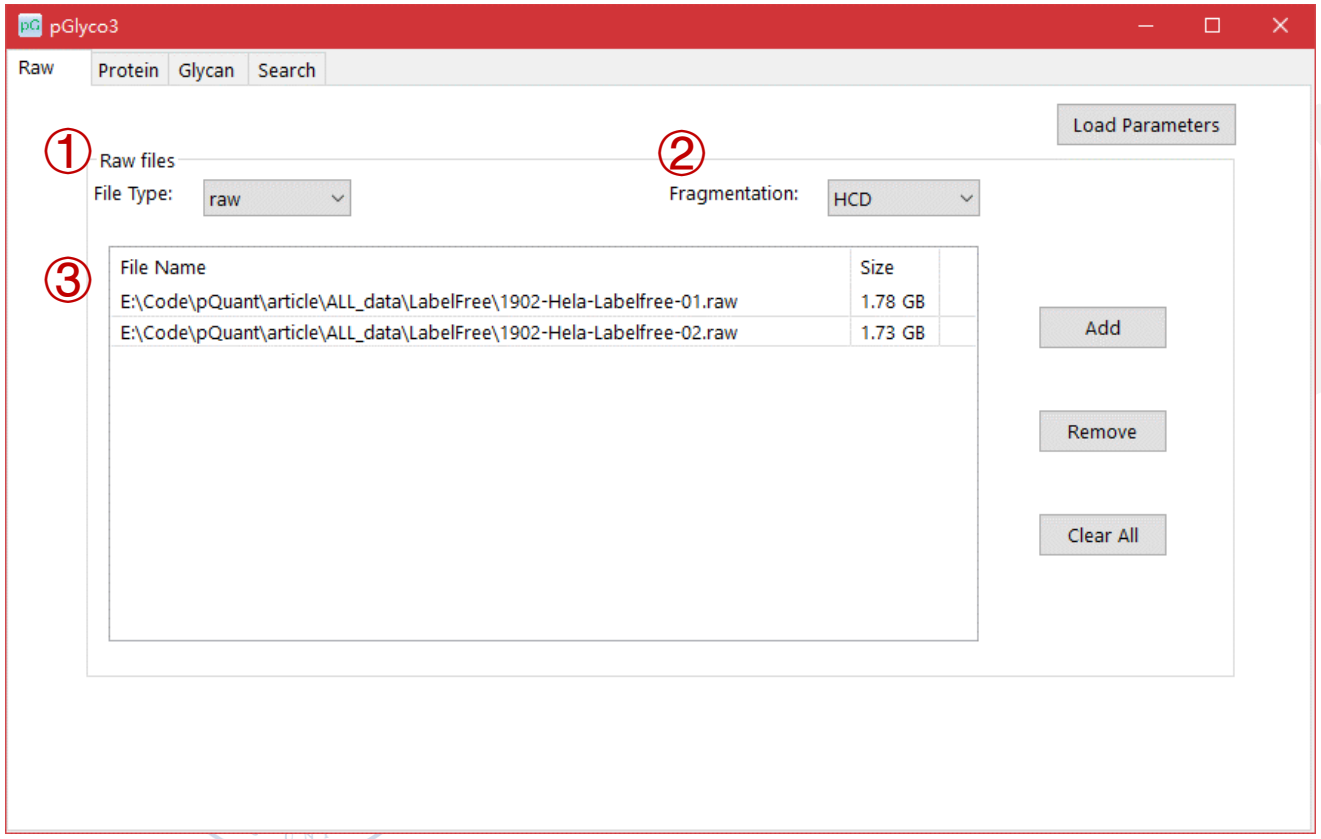
pGlyco & pGlycoQuant

Manual for DDA Label Free Data

1 Identification with pGlyco

1.1 pGlyco identification

Label Free



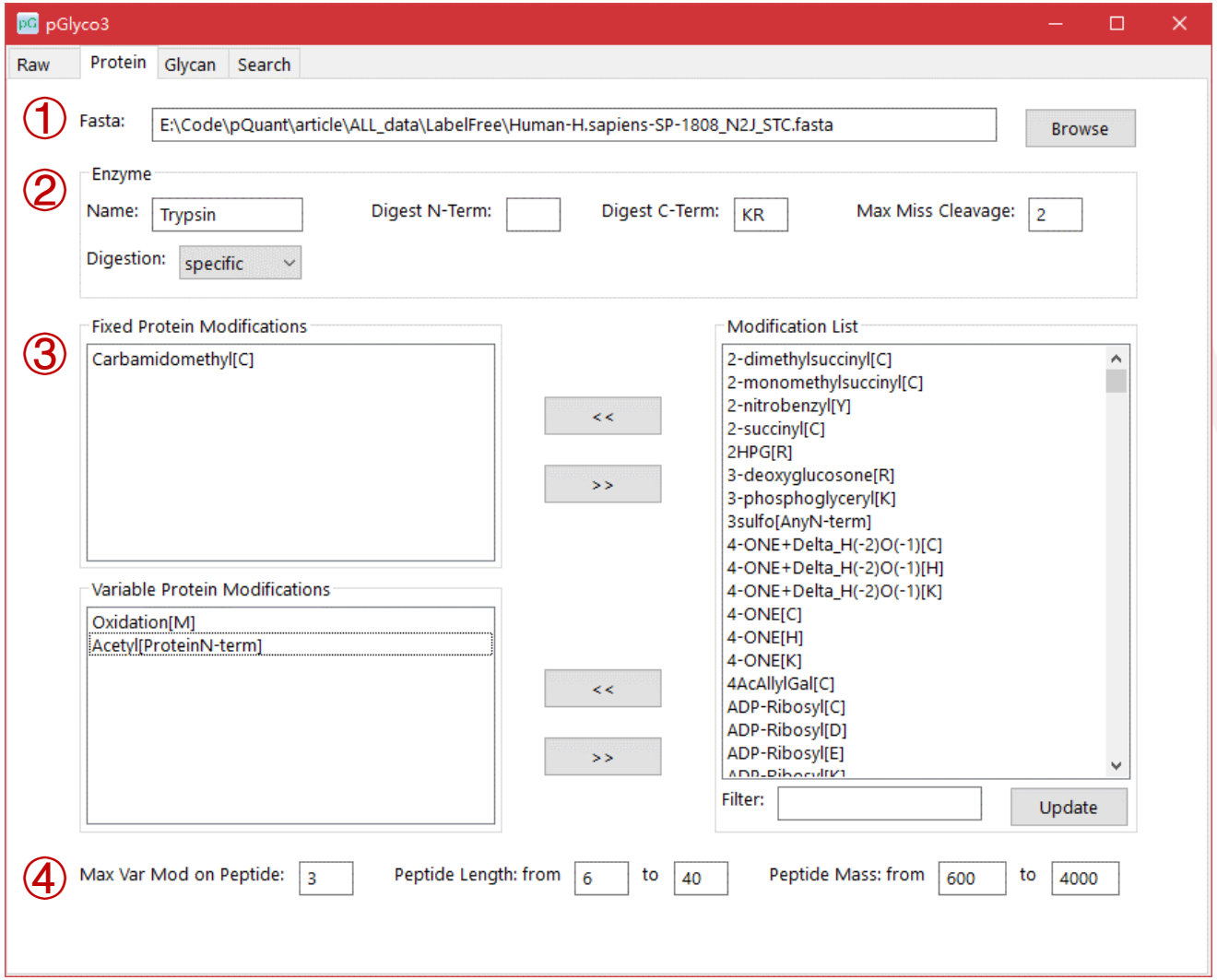
① Load raw files.

② Set the **Fragmentation** as **HCD**.

③ Add the **Label Free** file.

1 Identification with pGlyco

1.1 pGlyco identification



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

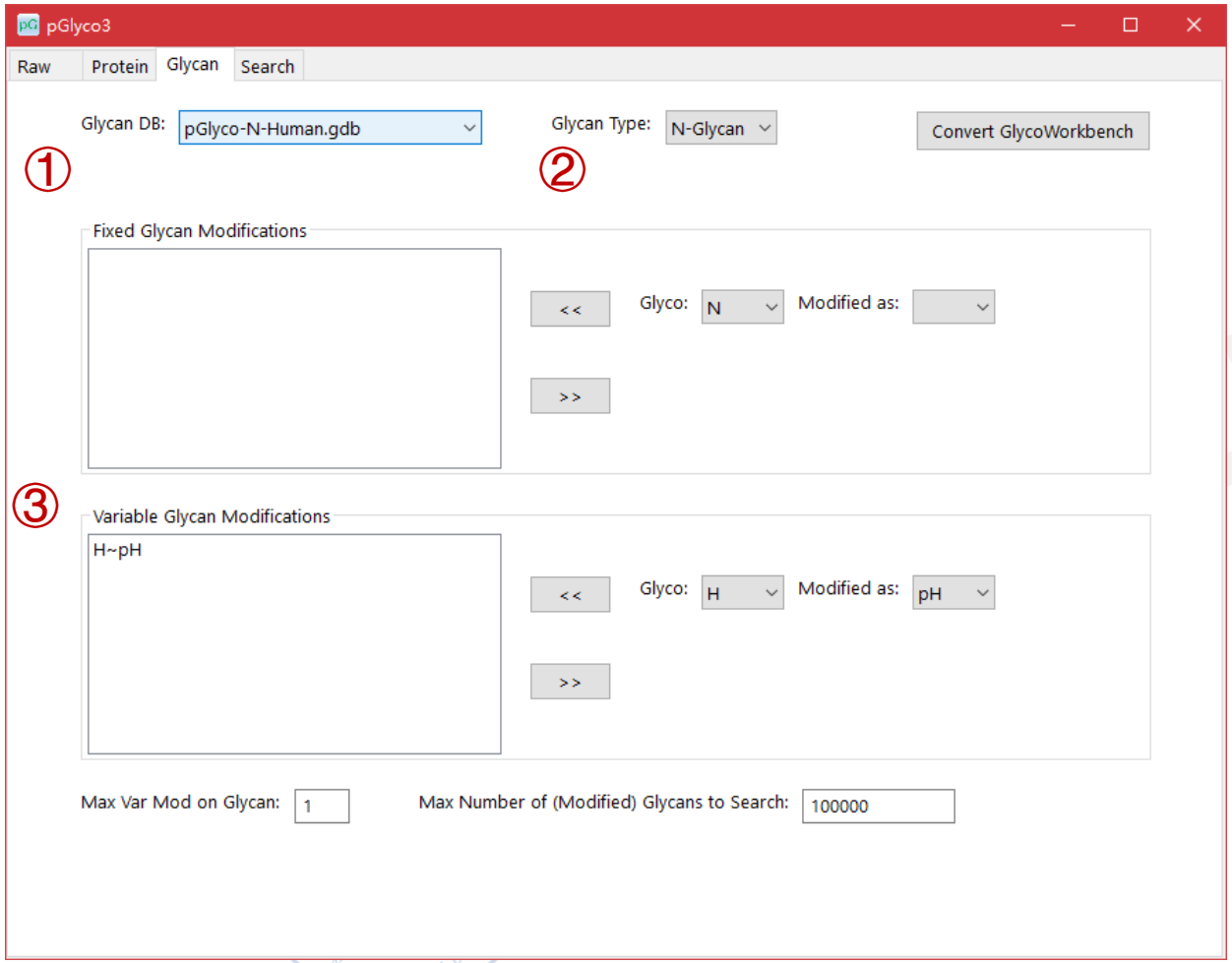
② Set the trypsin enzyme.

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



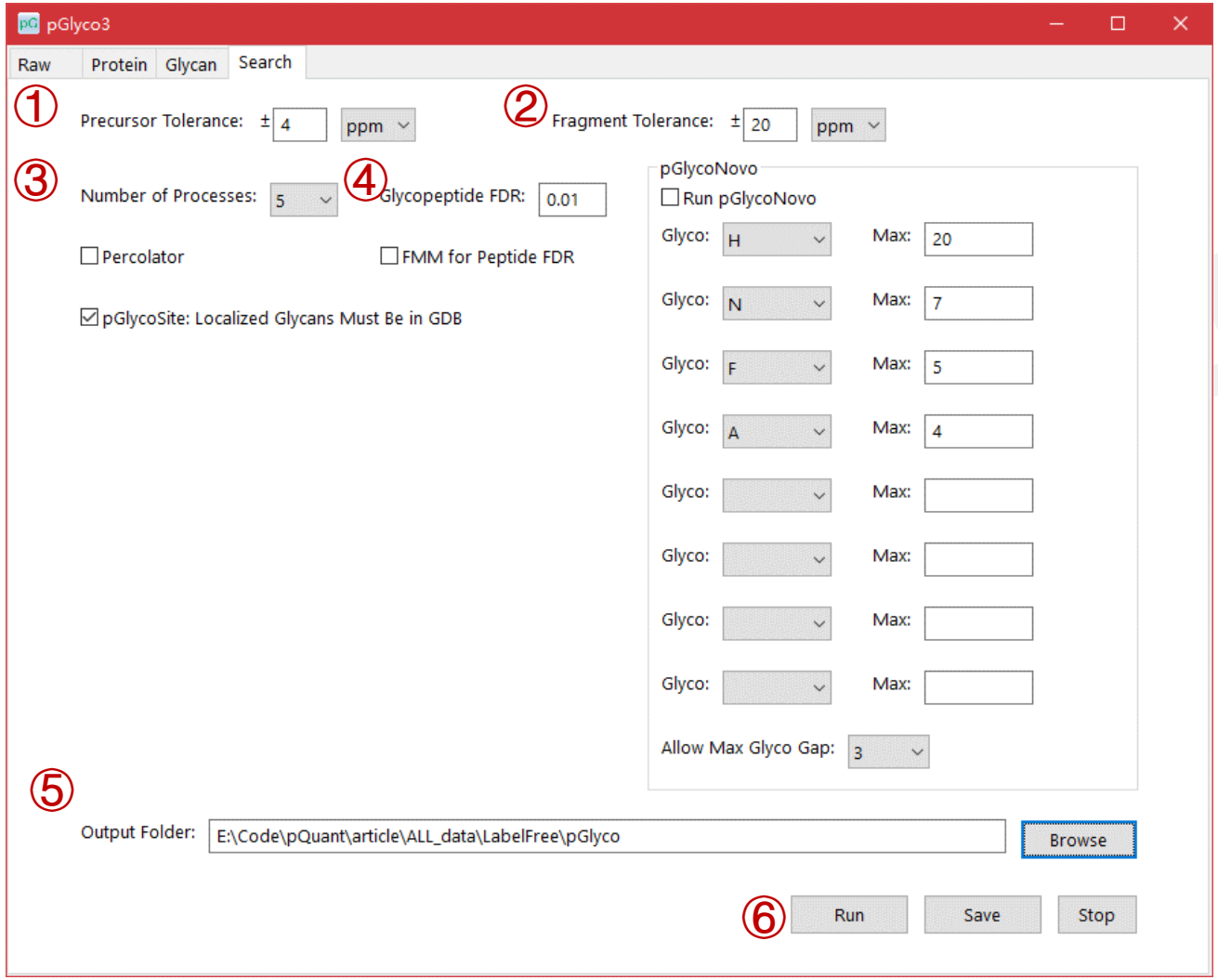
① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.

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

③ Set the Glycan modification information.

1 Identification with pGlyco

1.1 pGlyco identification



① Set the **Precursor Tolerance** as ±**4** ppm.

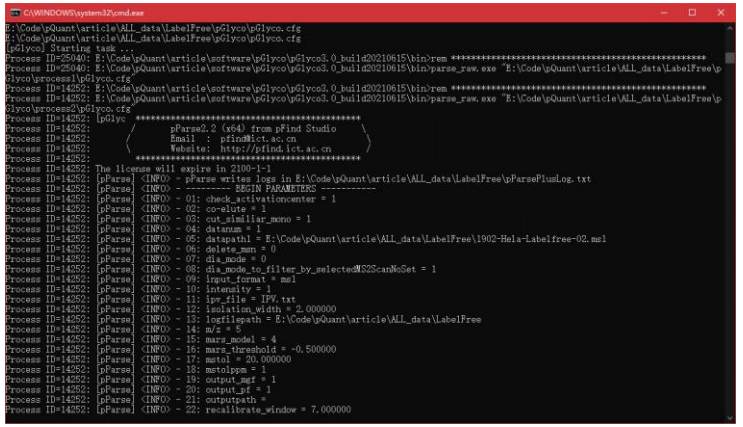
② Set the **Fragment Tolerance** as ±**20** ppm.

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.

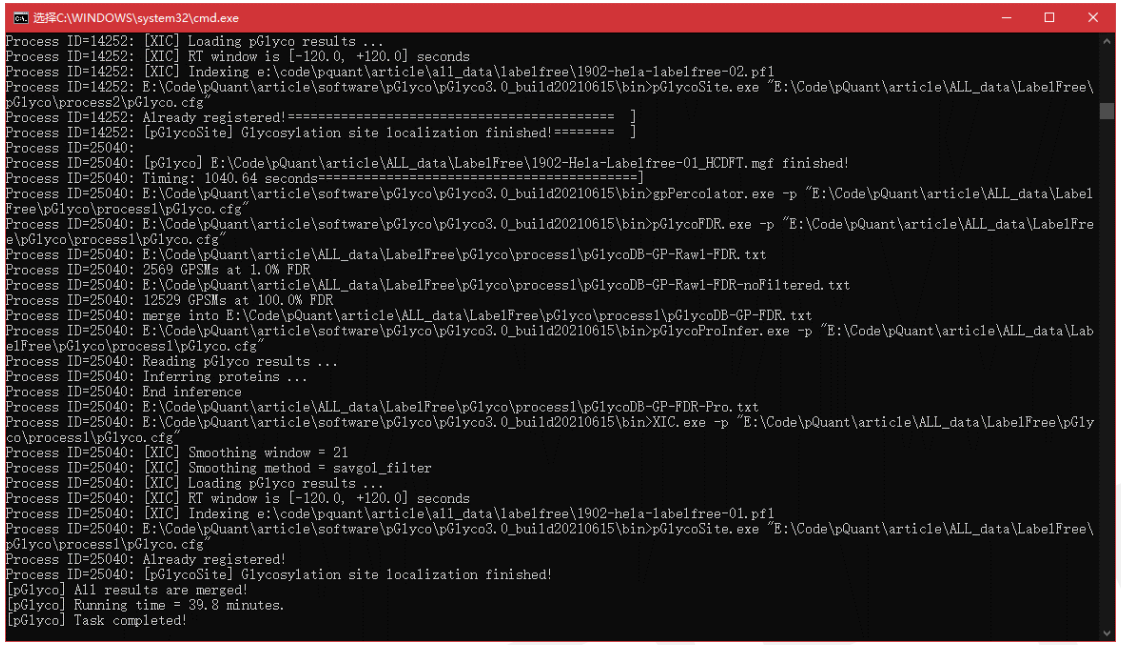


1 Identification with pGlyco

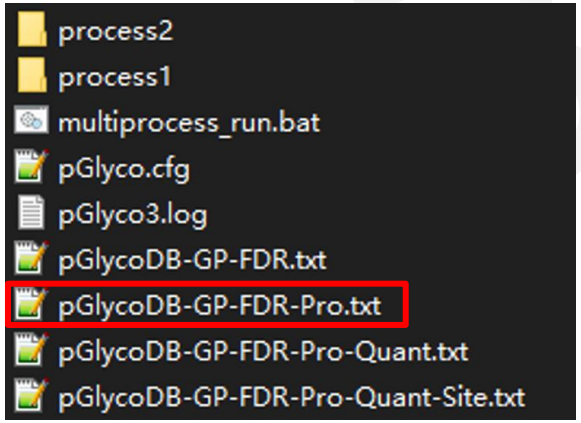
1.2 identification results

Label Free

①



②



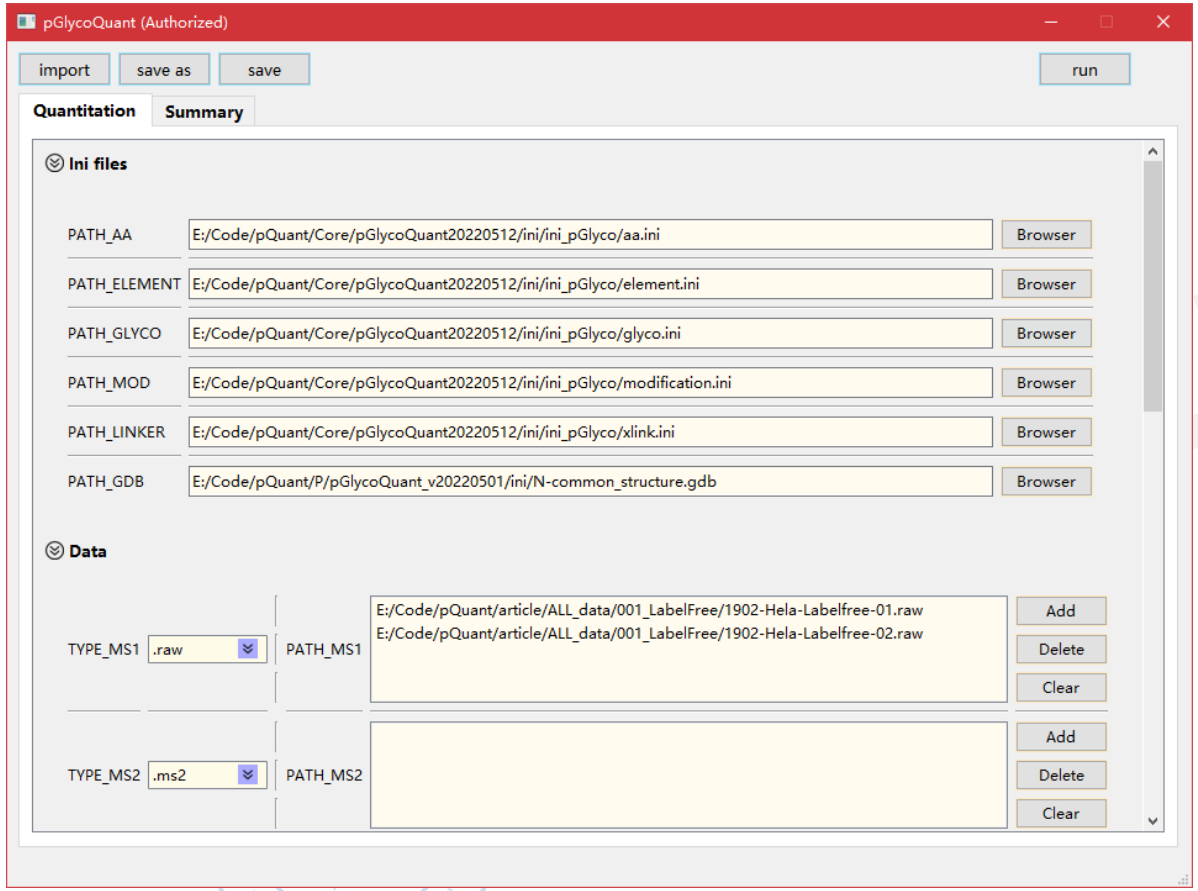
① The completed information in the command-line interface.

② The identification result file used for quantitation.

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation

Label Free



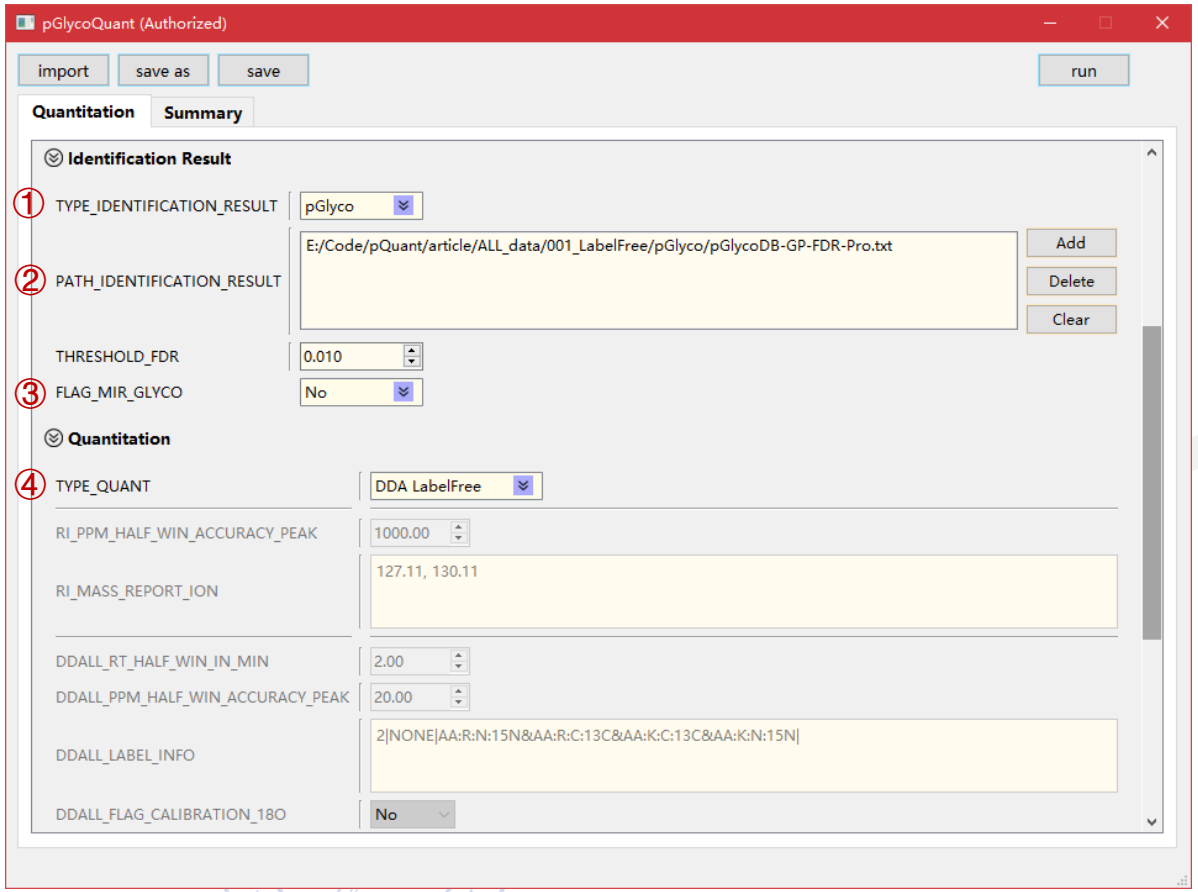
① Ensure that the ini file paths are valid.

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



① Set **TYPE_IDENTIFICATION_RESULT** as **pGlyco** (For other identification software results like Byonic and MSFragger, Byonic and MSFragger glyco-N options also can be chosen).

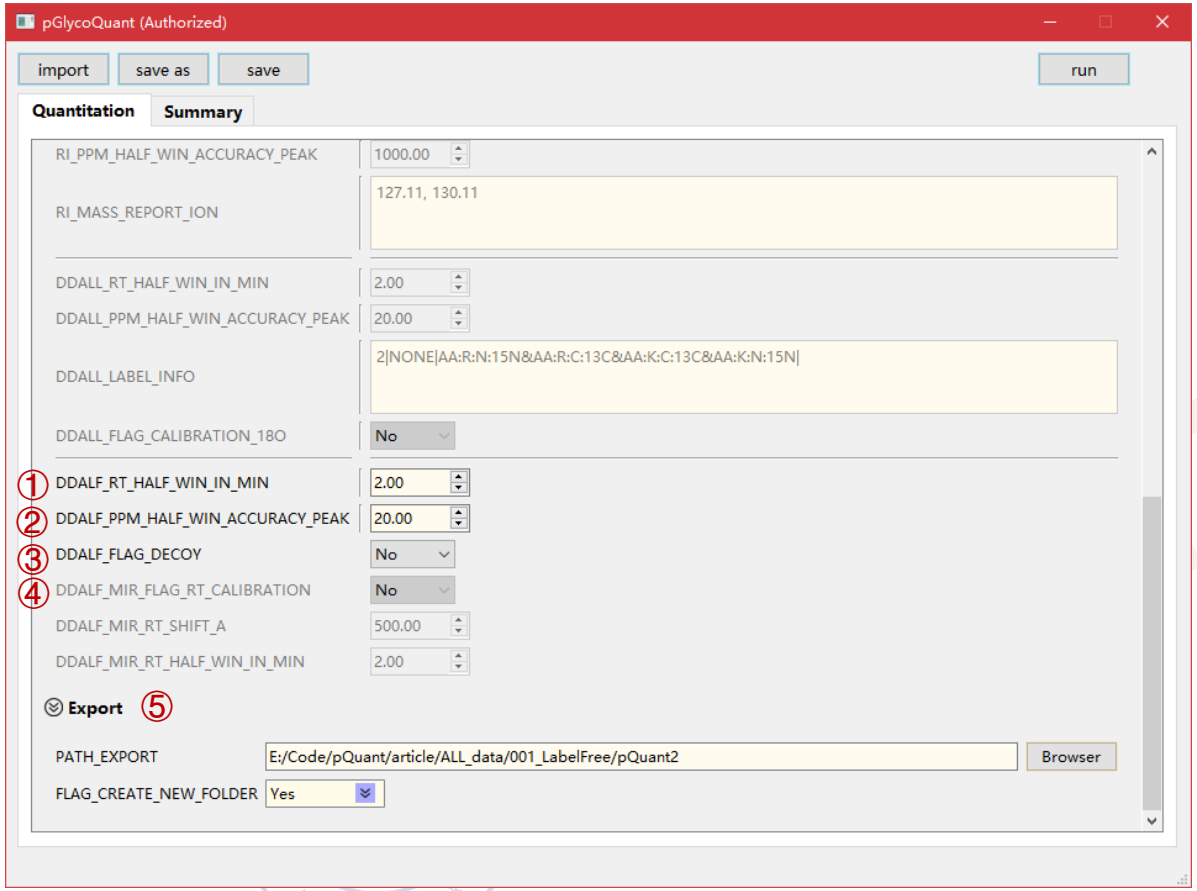
② Put the identification result file **pGlycoDB-GP-FDR-Pro.txt** here and set **FDR** as **0.01**.

③ **If MIR quantitation is needed, the FLAG_MIR_GLYCO can be set as Yes.**

④ Set the **TYPE_QUANT** as **DDA LabelFree**.

2 Quantitation with pGlycoQuant

2.1 pGlycoQuant quantitation



Label Free

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

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

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

④ **If FLAG_MIR_GLYCO is set as Yes:**

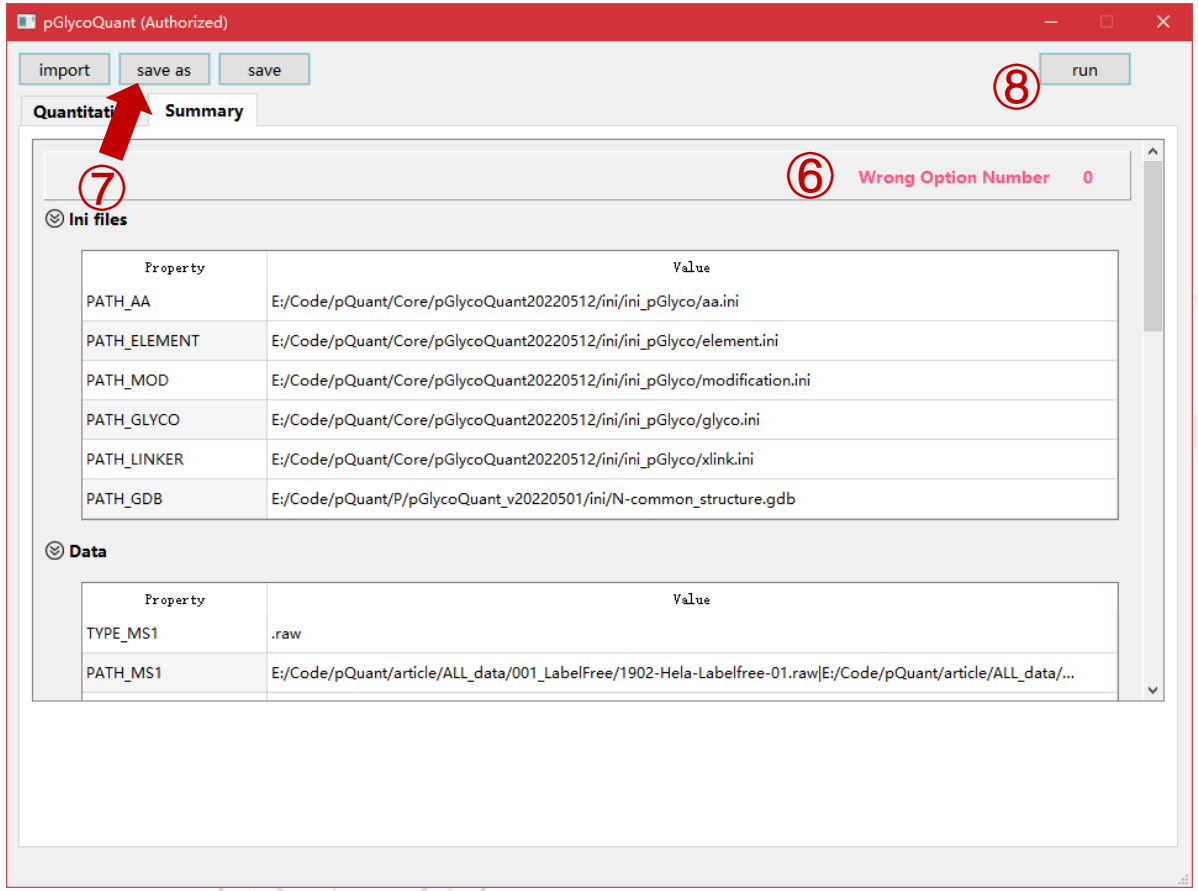
DDALF_MIR_FLAG_RT_CALIBRATION means the switch for manually adjusting RT_{SA}, if "Yes" was chosen, DDALF_MIR_RT_SHIFT_A (second) should be set.

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

⑤ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

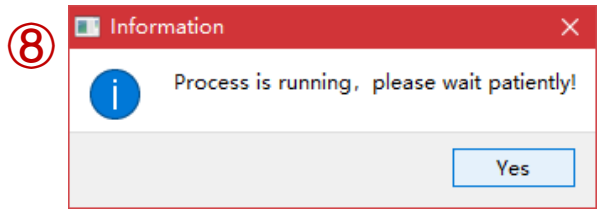
2.1 pGlycoQuant quantitation

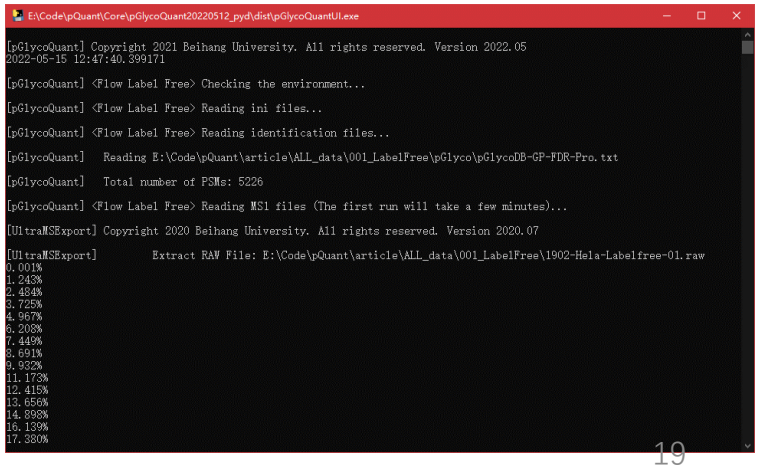


⑥ Click **Summary** button and make sure that the **Wrong Option Number** is **0**.

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

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



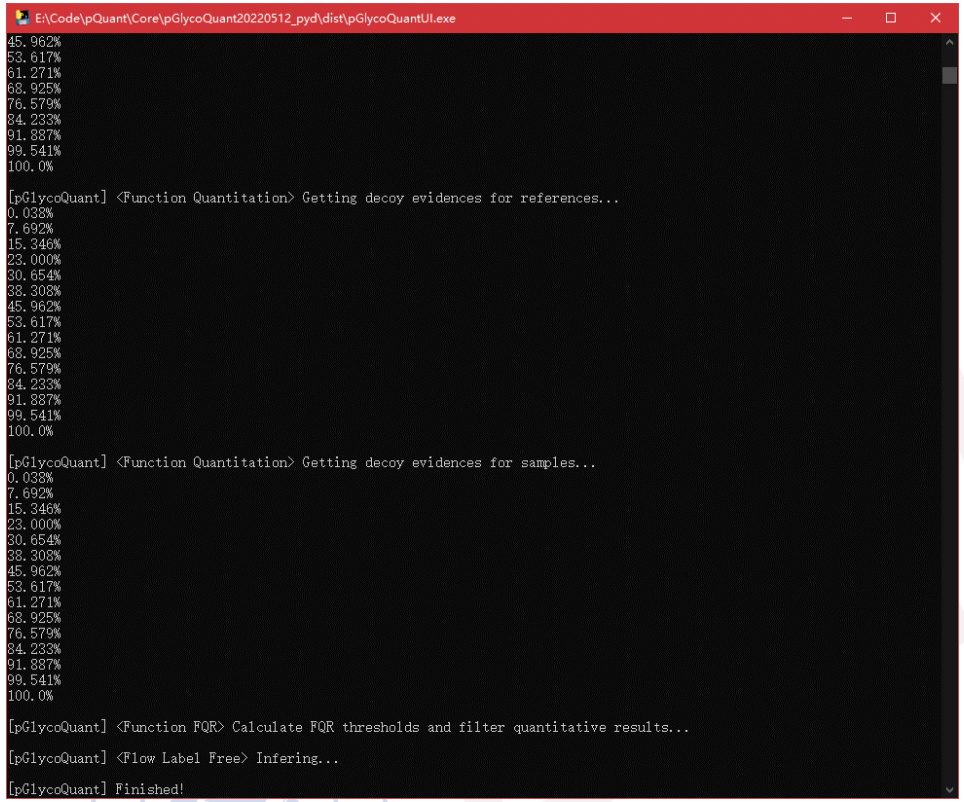


2 Quantitation with pGlycoQuant

2.2 quantitation results

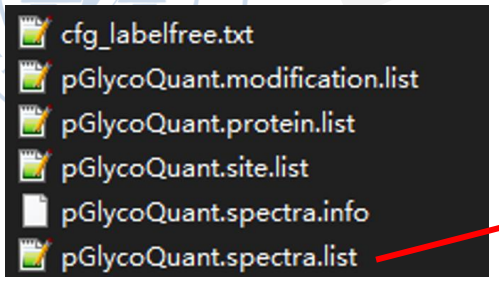
Label Free

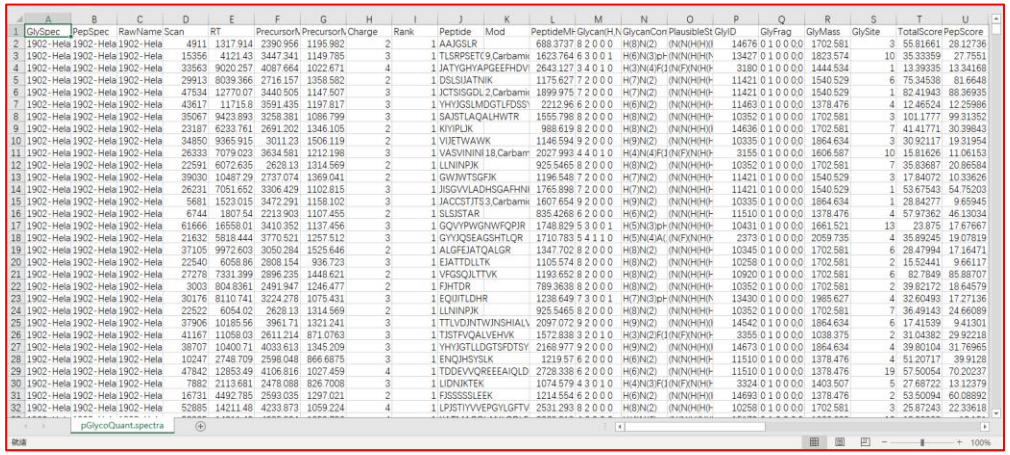
①



② The completed information.

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

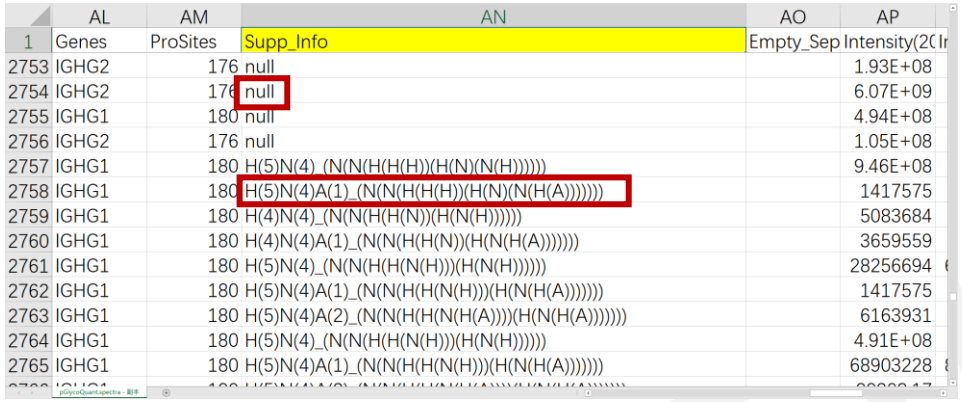




2 Quantitation with pGlycoQuant

2.2 quantitation results --- MIR results

Label Free



	AL	AM	AN	AO	AP
1	Genes	ProSites	Supp_Info	Empty_Sep	Intensity(2(
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(A))))(H(N(H(A)))))))		6163931
2764	IGHG1	180	H(5)N(4)_(N(N(H(H(N(H)))(H(N(H))))))		4.91E+08
2765	IGHG1	180	H(5)N(4)A(1)_(N(N(H(H(N(H)))(H(N(H(A)))))))		68903228

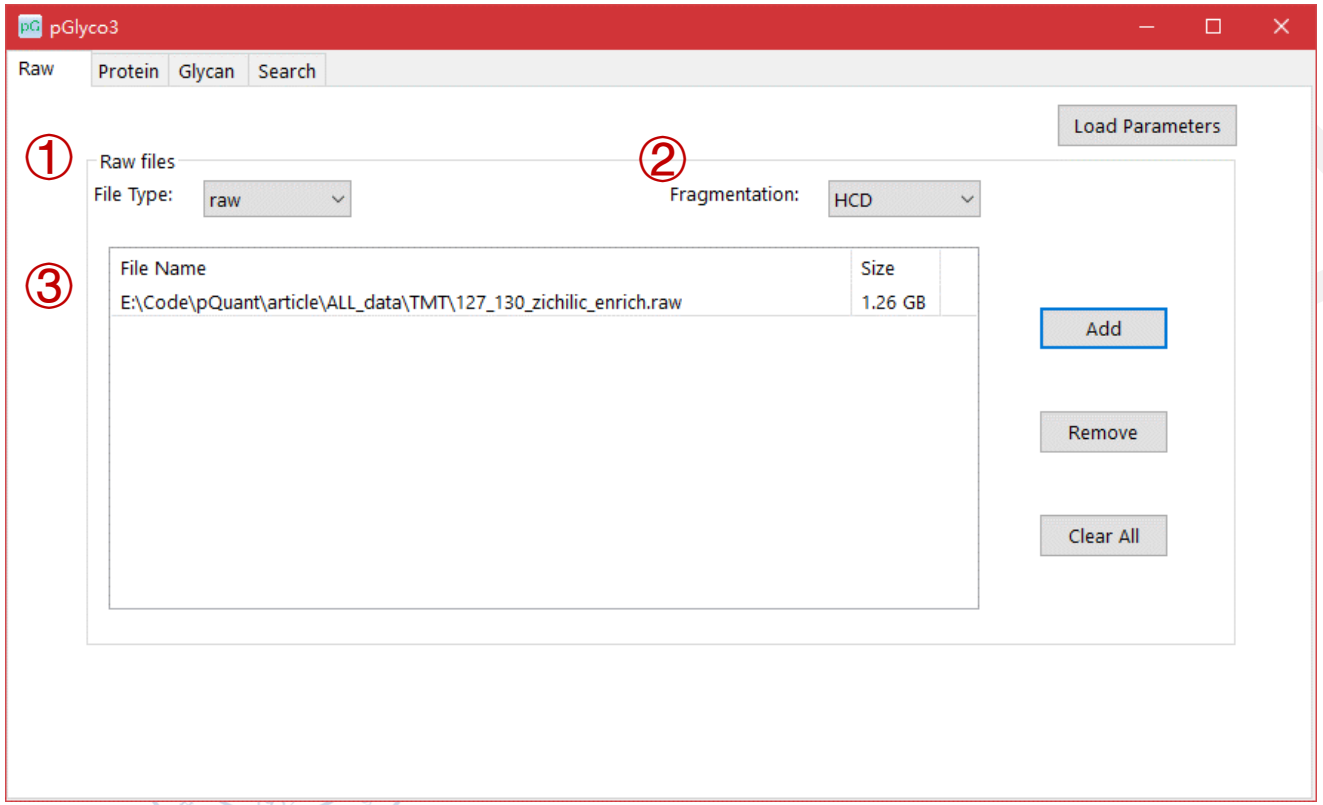
The “Supp_Info” column shows the information of MIR quantitation: “null” means the quantitation of identified result from pGLyco, and “ H(5)N(4)A(1)_(N(N(H(H(H))(H(N)(N(H(A))))))) ” means the quantitated candidate glycopeptide from the subnet.

pGlyco & pGlycoQuant

Manual for TMT Data

1. Identification with pGlyco

TMT



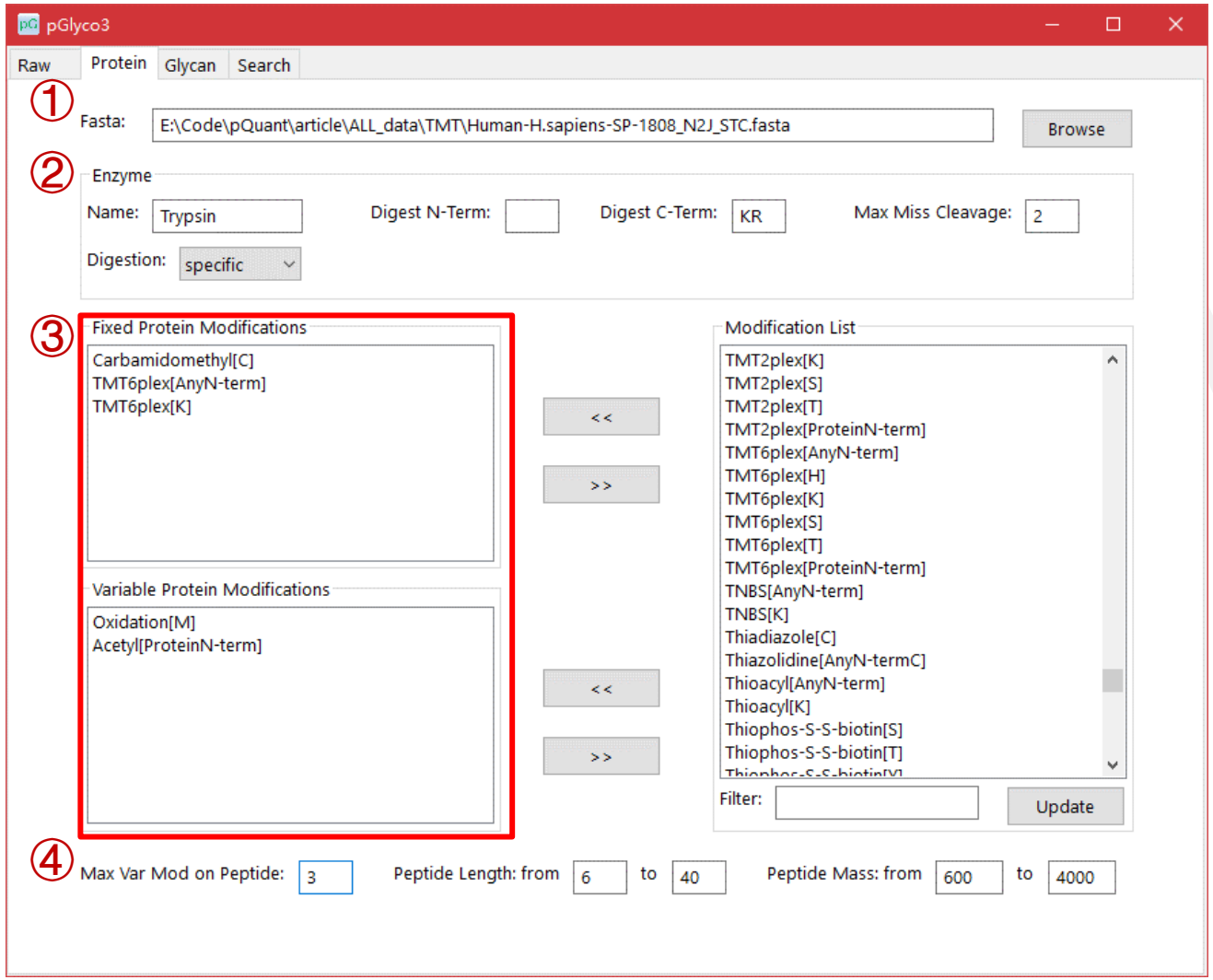
① Load raw files.

② Set the **Fragmentation** as **HCD**.

③ Add the **TMT** raw file.

1. Identification with pGlyco

TMT



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

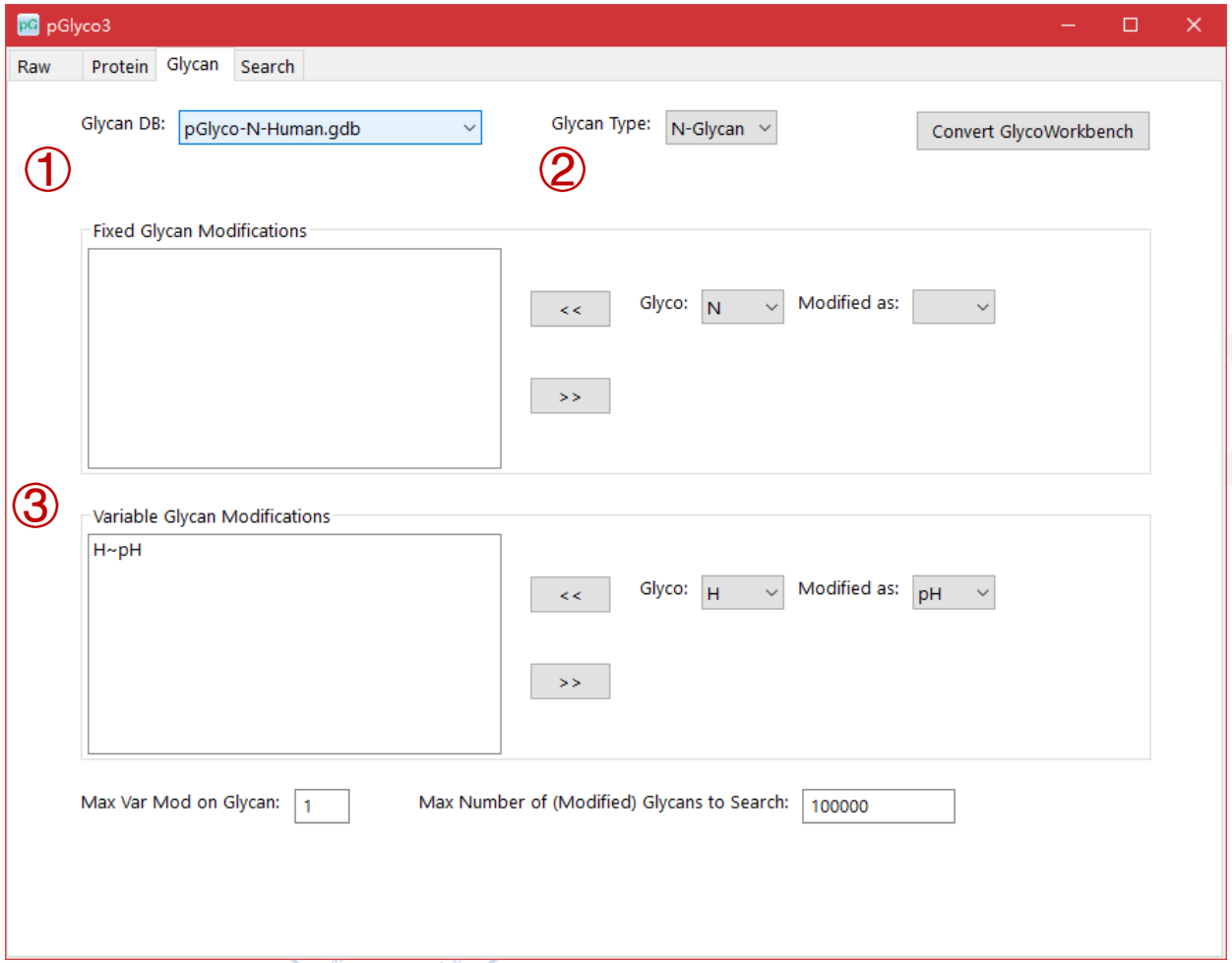
② Set the trypsin enzyme.

③ Set the modification information like the left panel.

④ Set the filter information.

1. Identification with pGlyco

TMT

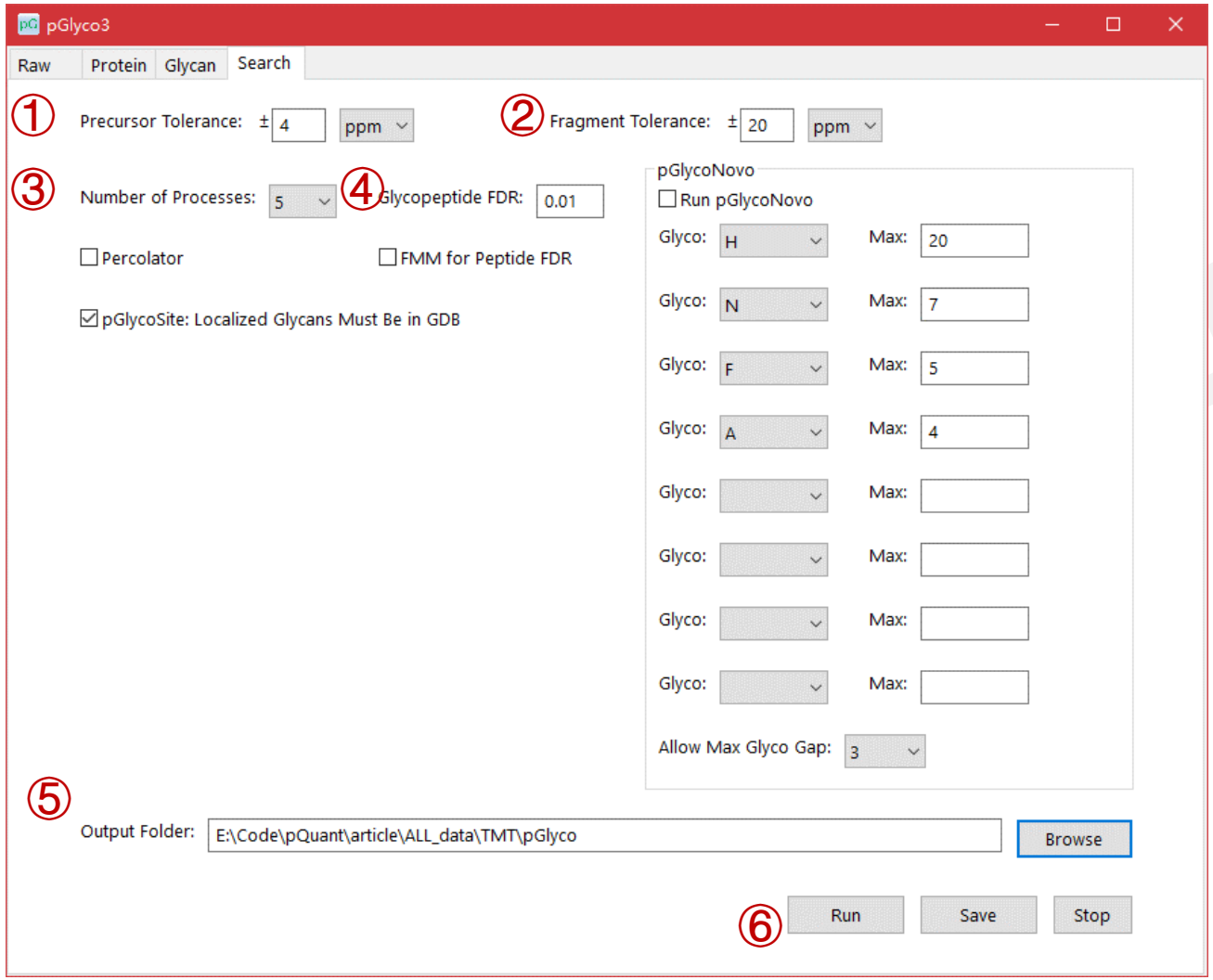


① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.

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

③ Set the Glycan modification information.

1. Identification with pGlyco



① Set the **Precursor Tolerance** as ±**4** ppm.

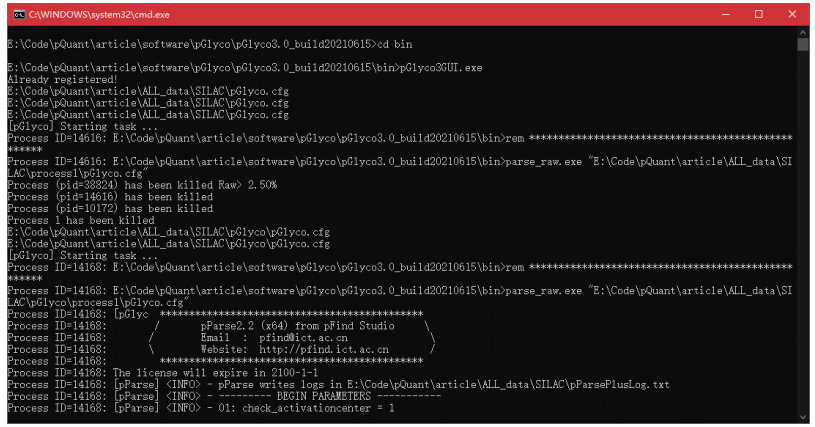
② Set the **Fragment Tolerance** as ±**20** ppm.

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.

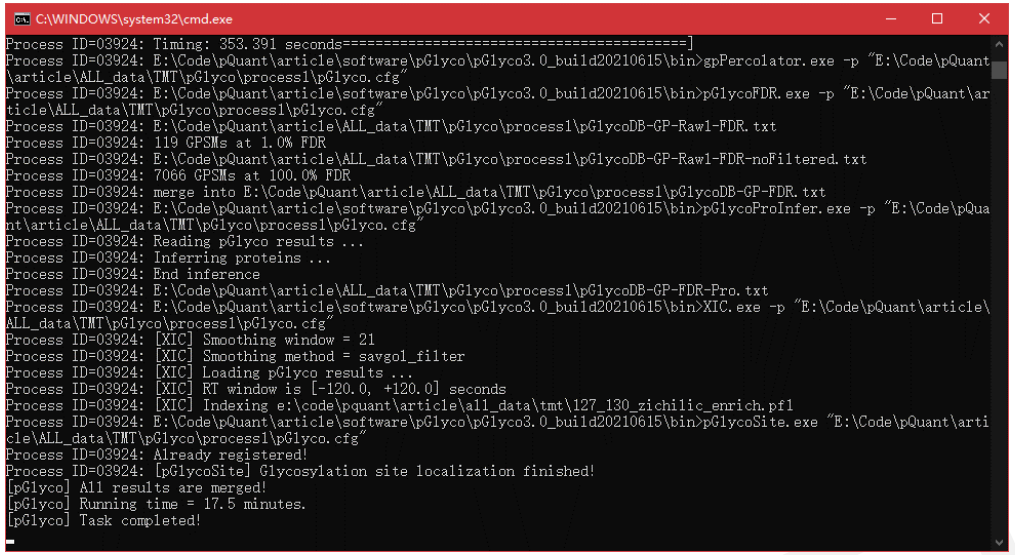
⑥ Click **Save** and **Run** buttons, the progress information will be shown in the command-line interface.



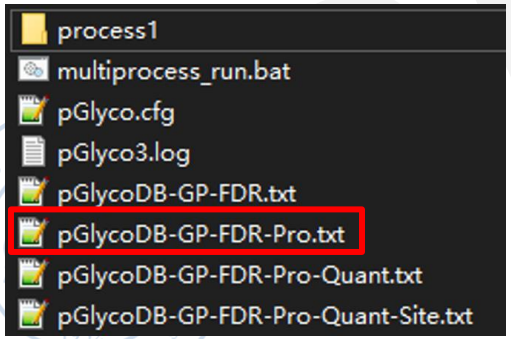
1. Identification with pGlyco

TMT

①



②



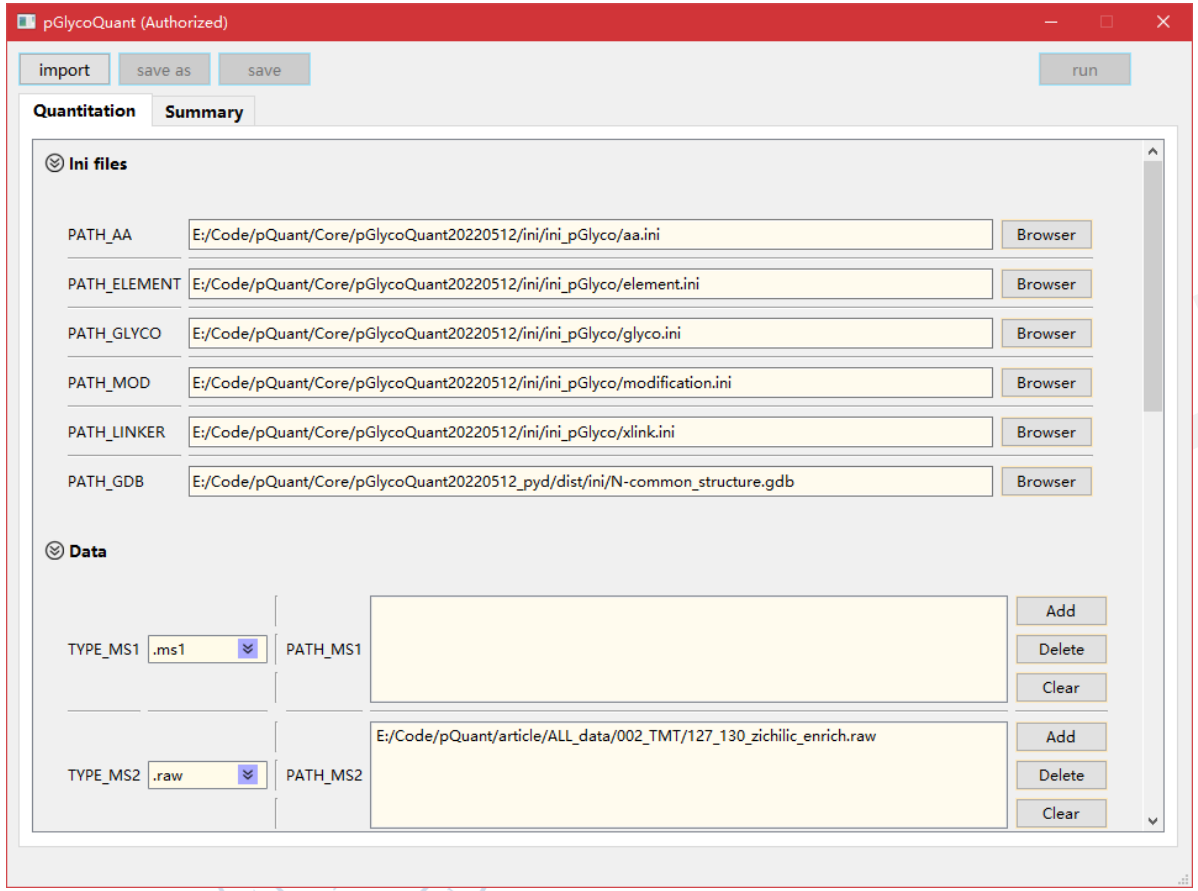
① The completed information in the command-line interface.

② The identification results.

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

TMT



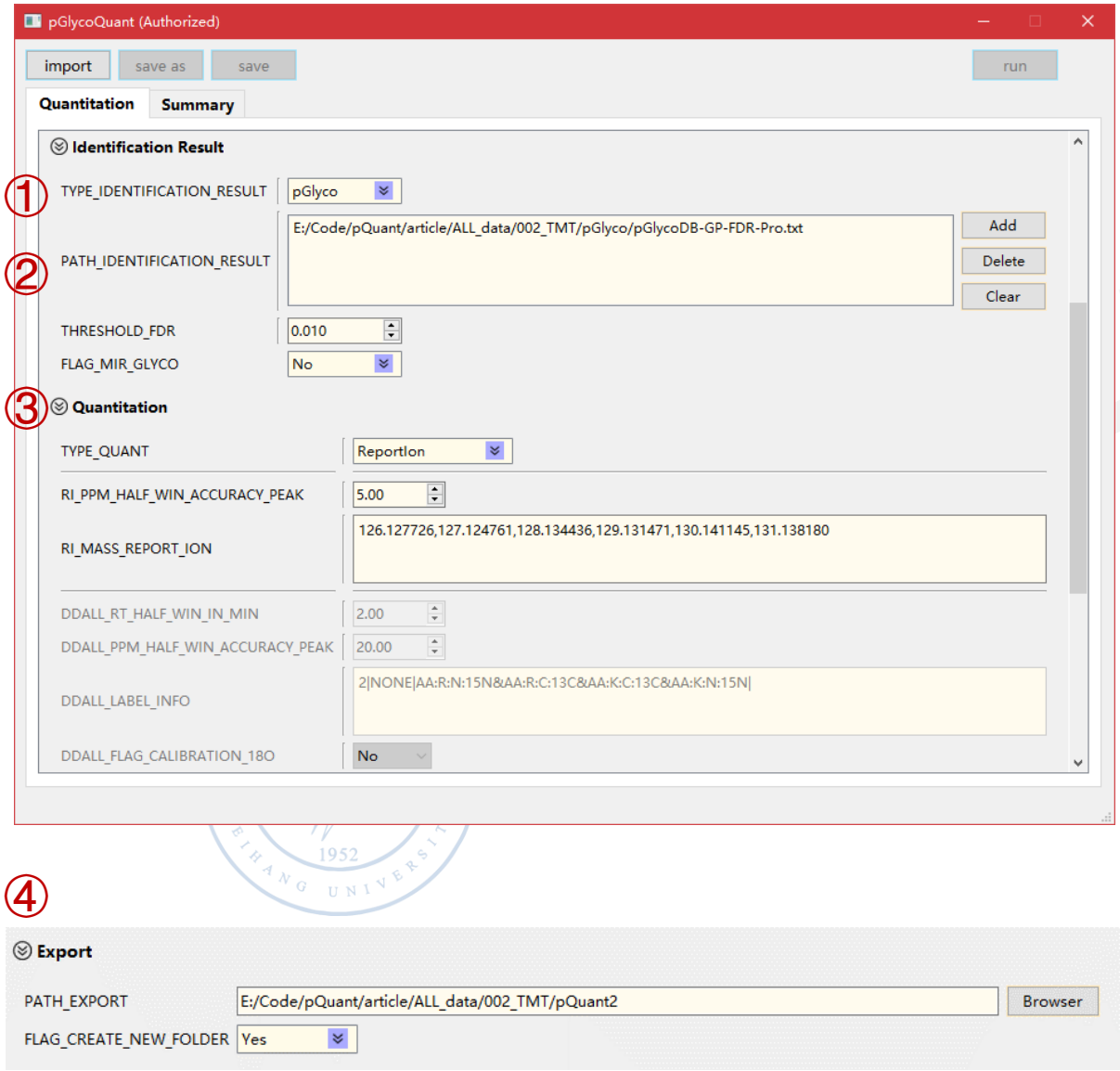
① Ensure that the ini file paths are valid.

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

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

TMT



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

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

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

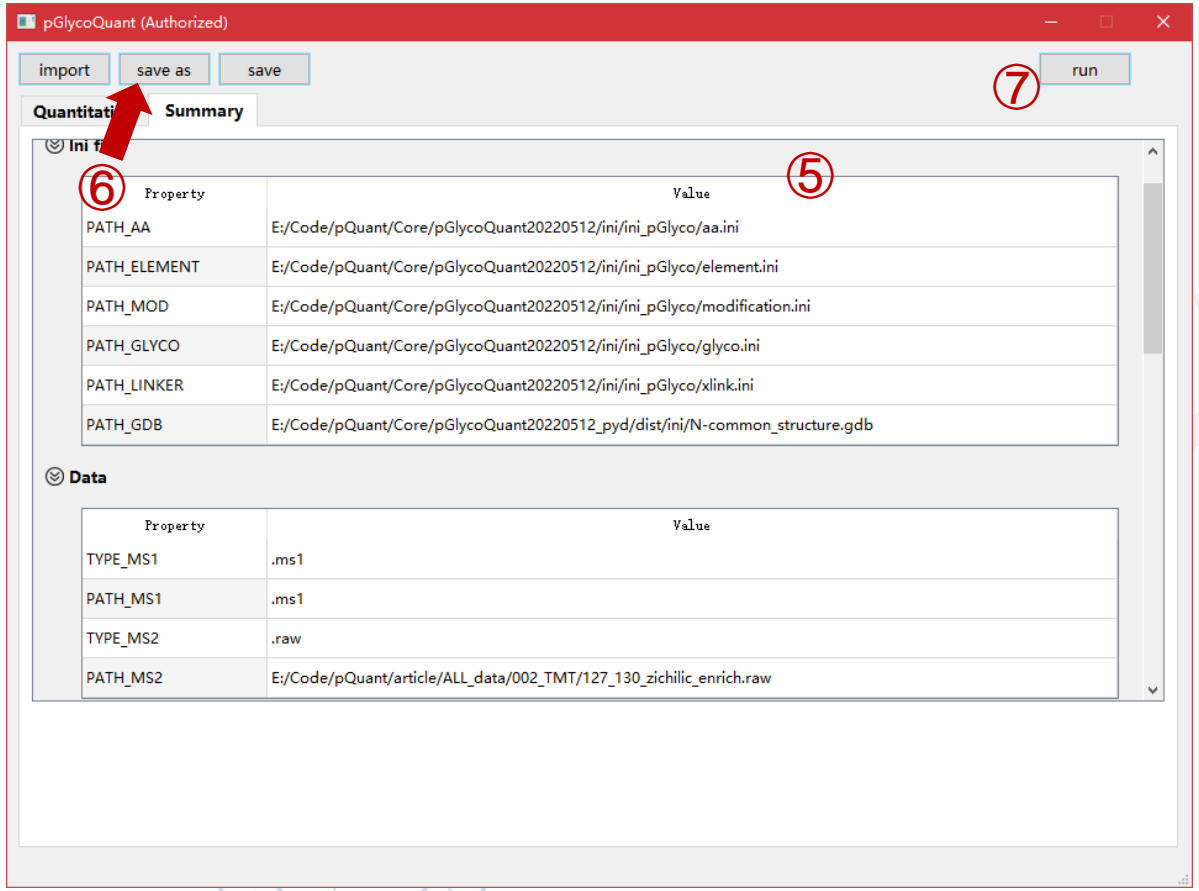
The **RI_MASS_REPORT_ION** could be **126.127726,127.124761,128.134436,129.131471,130.141145,131.138180**.

④ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

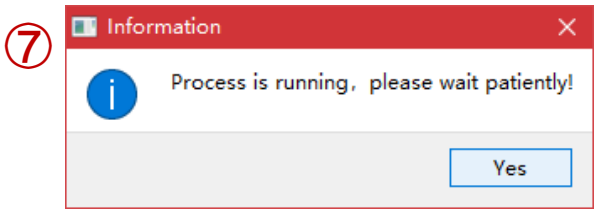
TMT

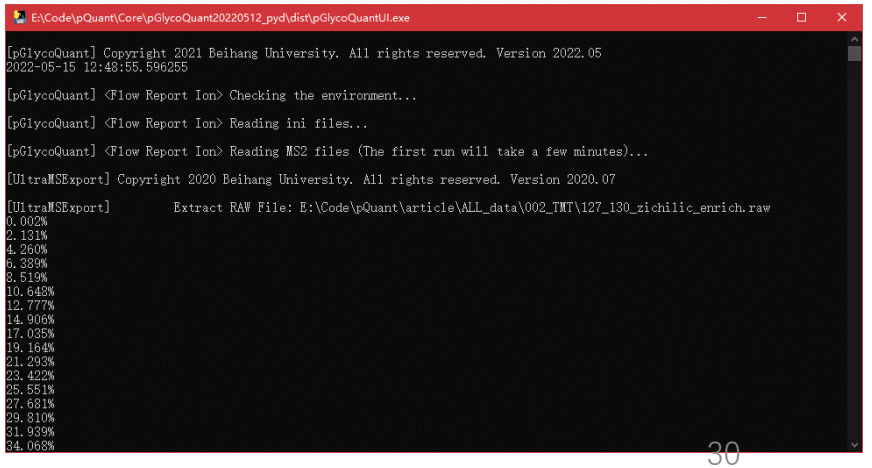


⑤ Click **Summary** button and make sure that the **Wrong Option Number** is **0**.

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

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



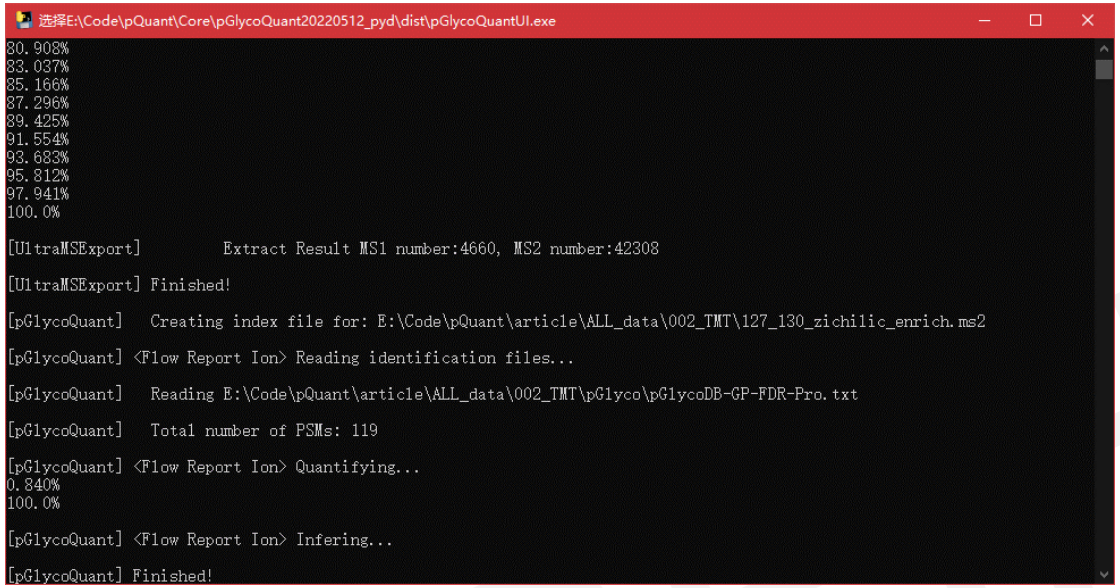


2 Quantitation with pGlycoQuant

1.4 quantitation results

TMT

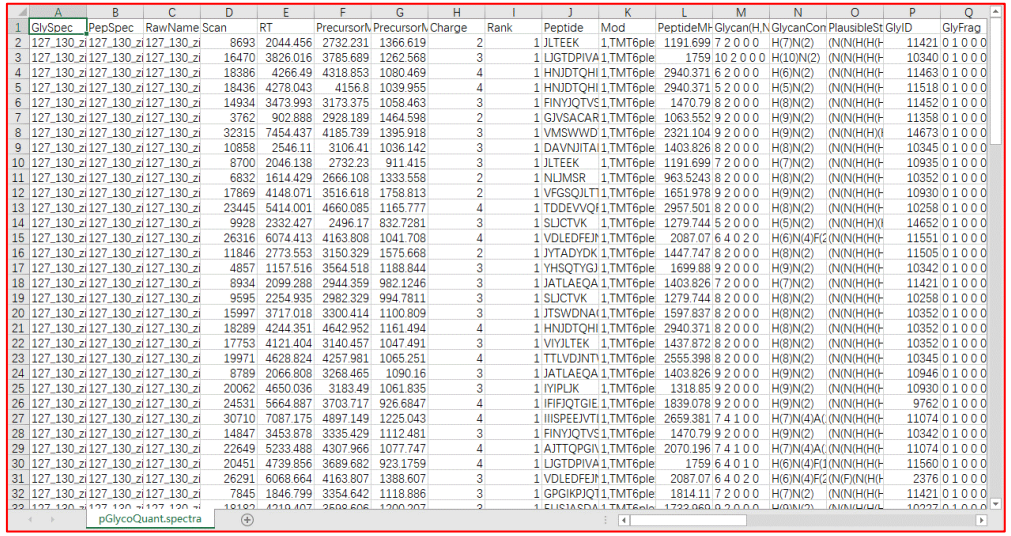
①



② cfg.txt
pGlycoQuant.modification.list
pGlycoQuant.protein.list
pGlycoQuant.site.list
pGlycoQuant.spectra.info
pGlycoQuant.spectra.list

① The completed information.

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

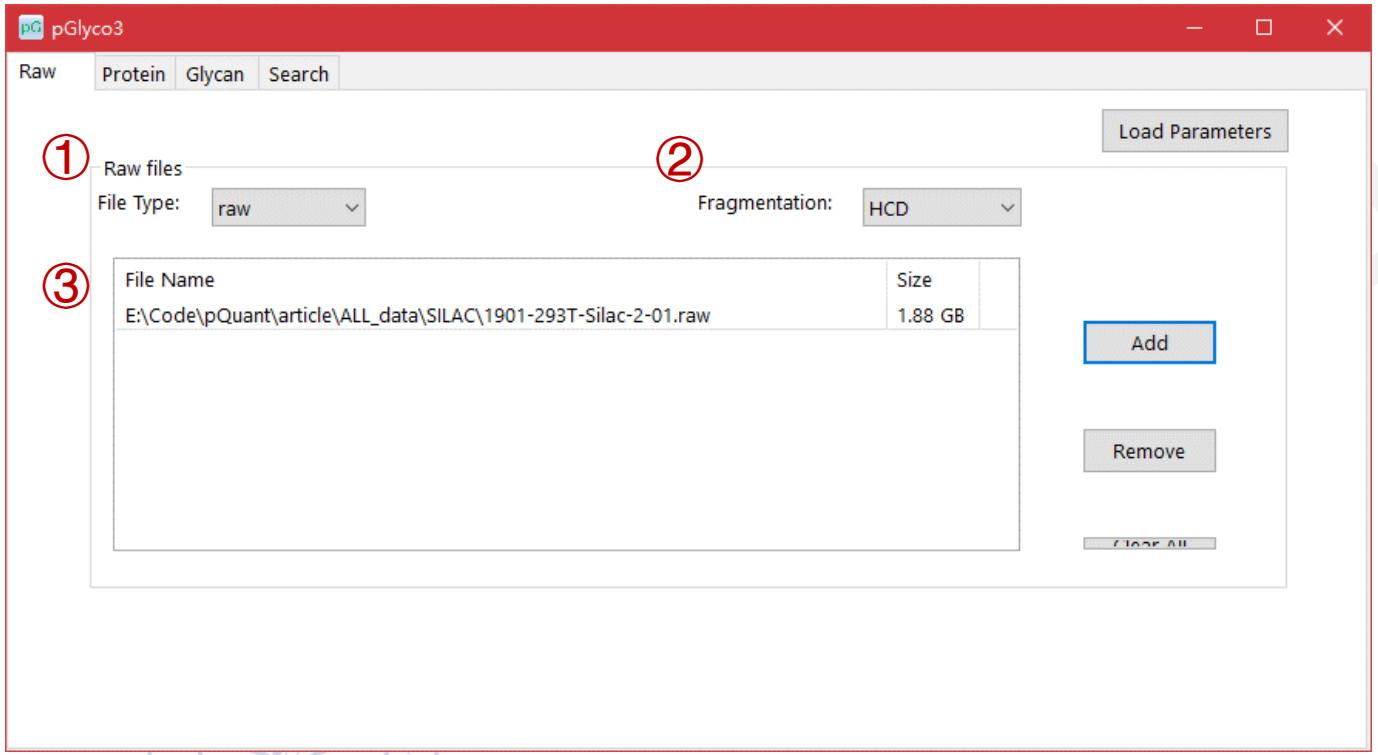


pGlyco & pGlycoQuant

Manual for SILAC Data

1. Identification with pGlyco

SILAC



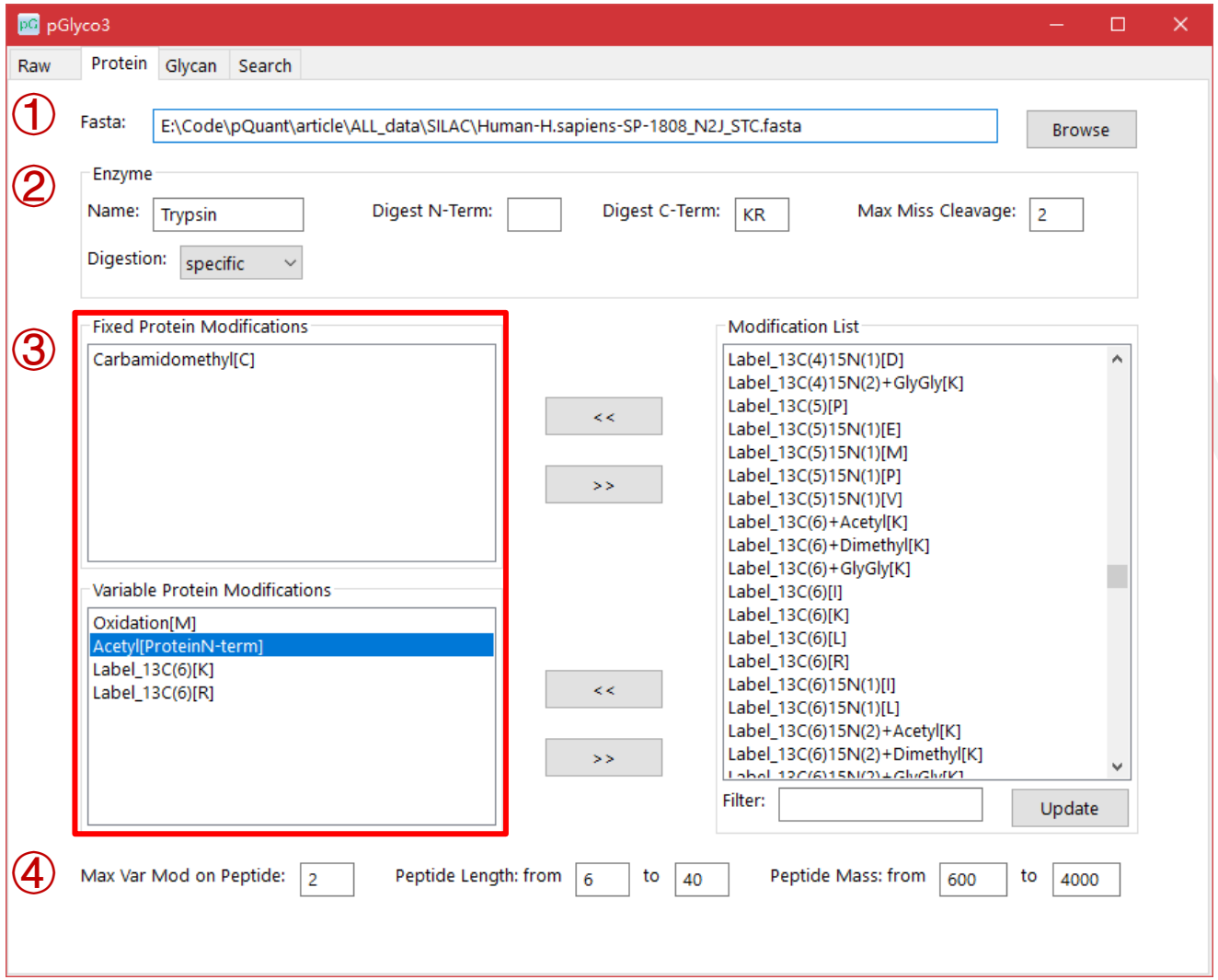
① Load raw files.

② Set the **Fragmentation** as **HCD**.

③ Add the **SILAC** raw file.

1. Identification with pGlyco

SILAC



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

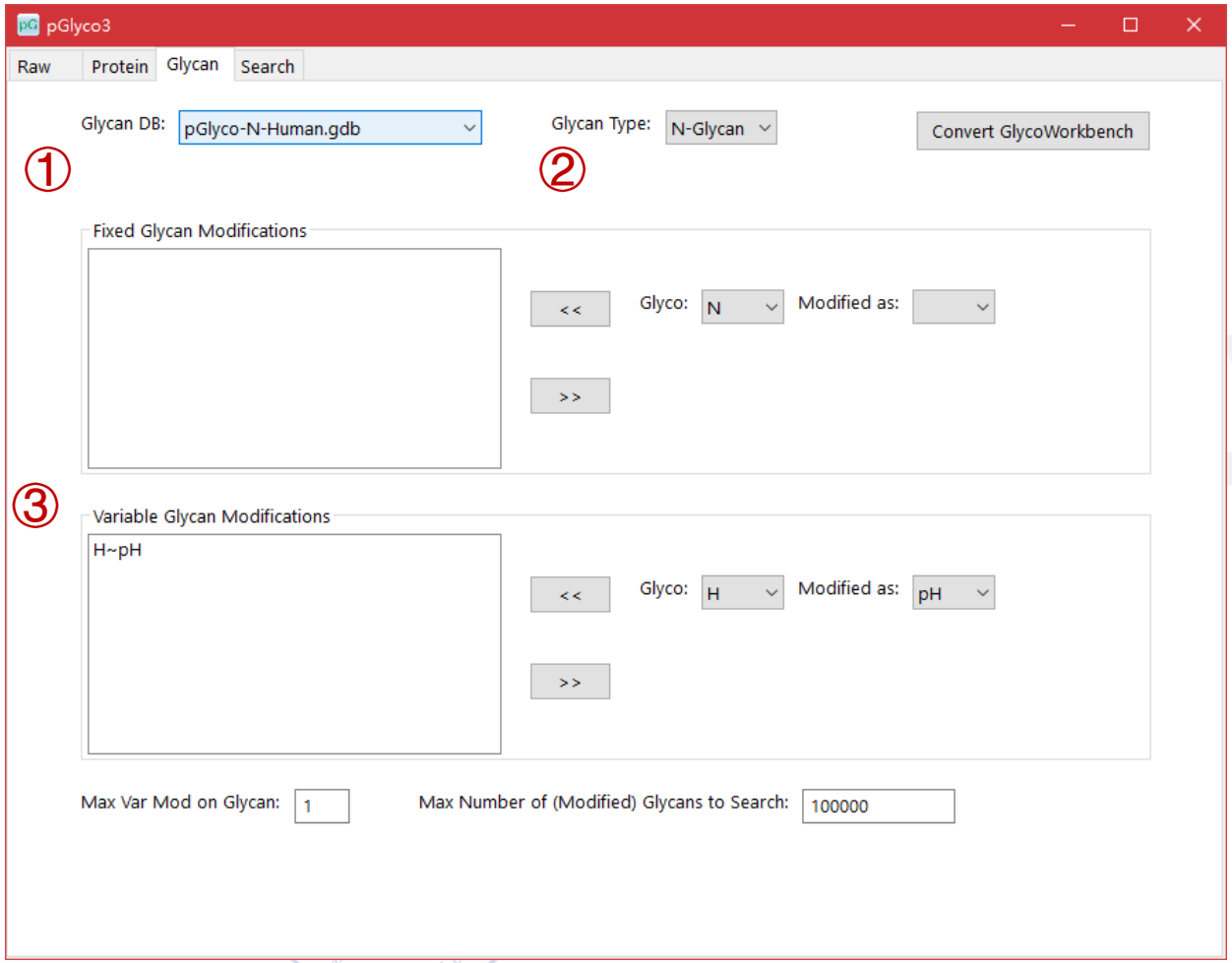
② Set the trypsin enzyme.

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



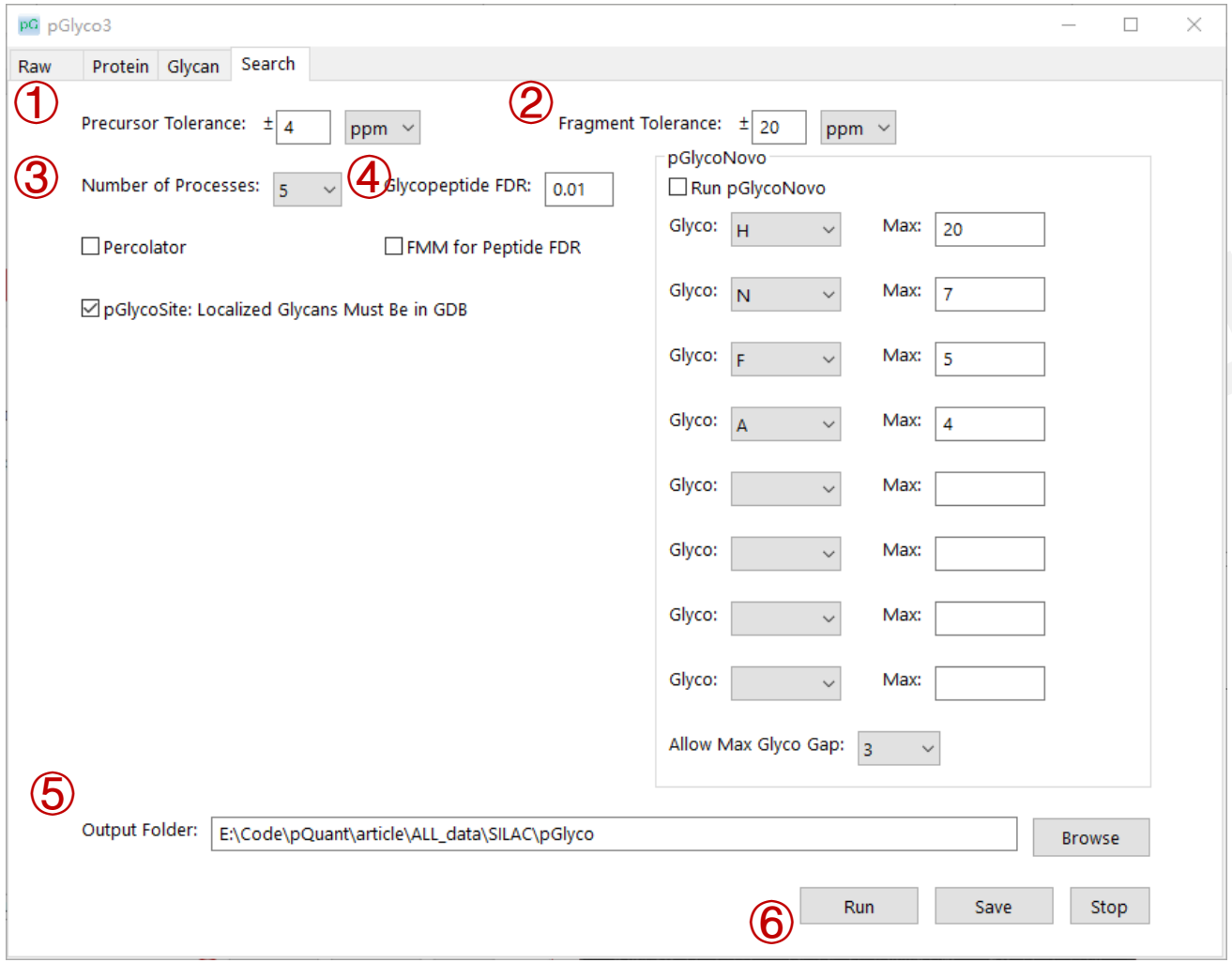
① Set the **Glycan DB** as **pGlyco-N-Human.gdb**.

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

③ Set the Glycan modification information.

1. Identification with pGlyco

SILAC



① Set the **Precursor Tolerance** as ±**4** ppm.

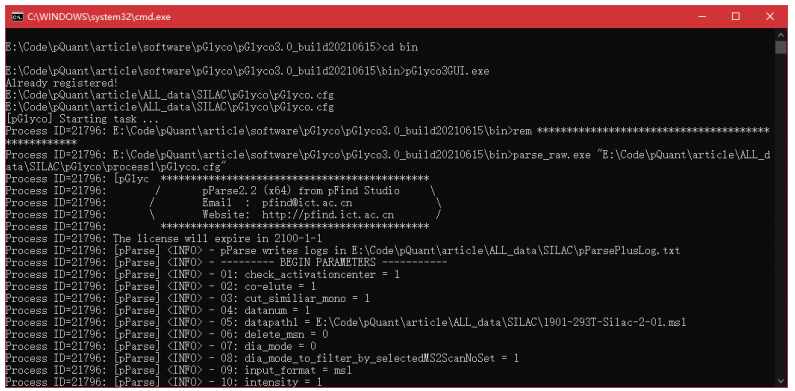
② Set the **Fragment Tolerance** as ±**20** ppm.

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.

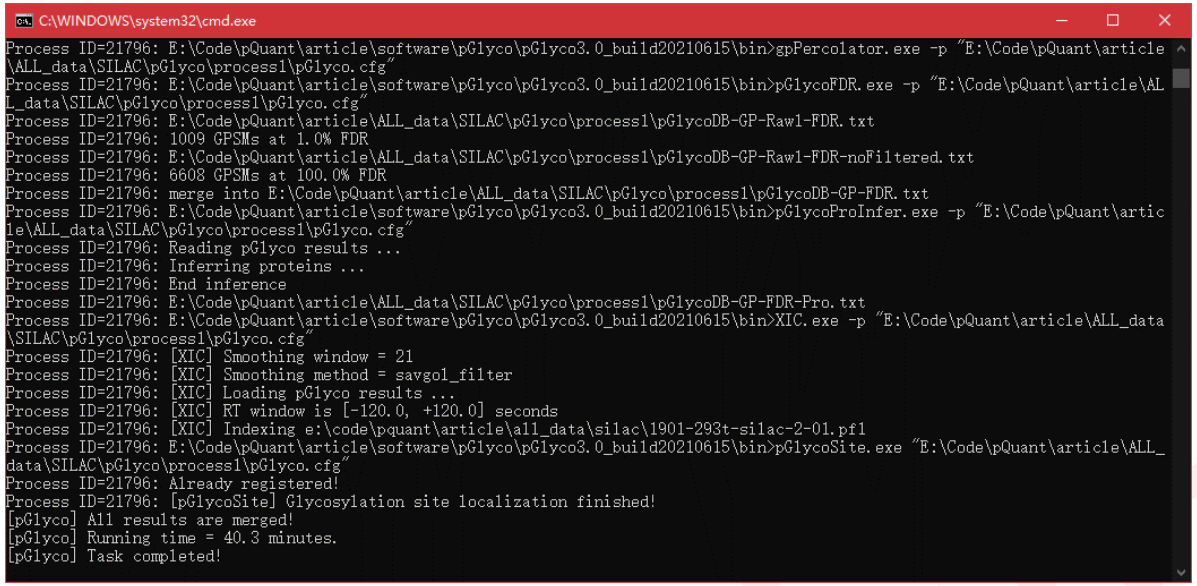
⑥ Click **Save** and **Run** buttons, the progress information will be shown in the command-line interface.



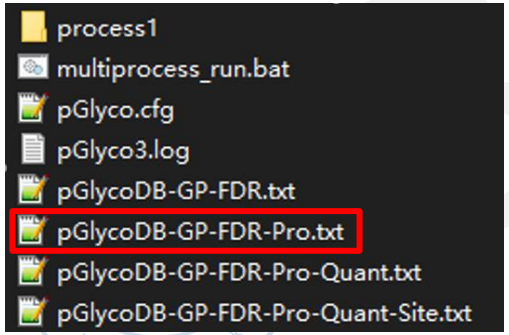
1. Identification with pGlyco

SILAC

①



②



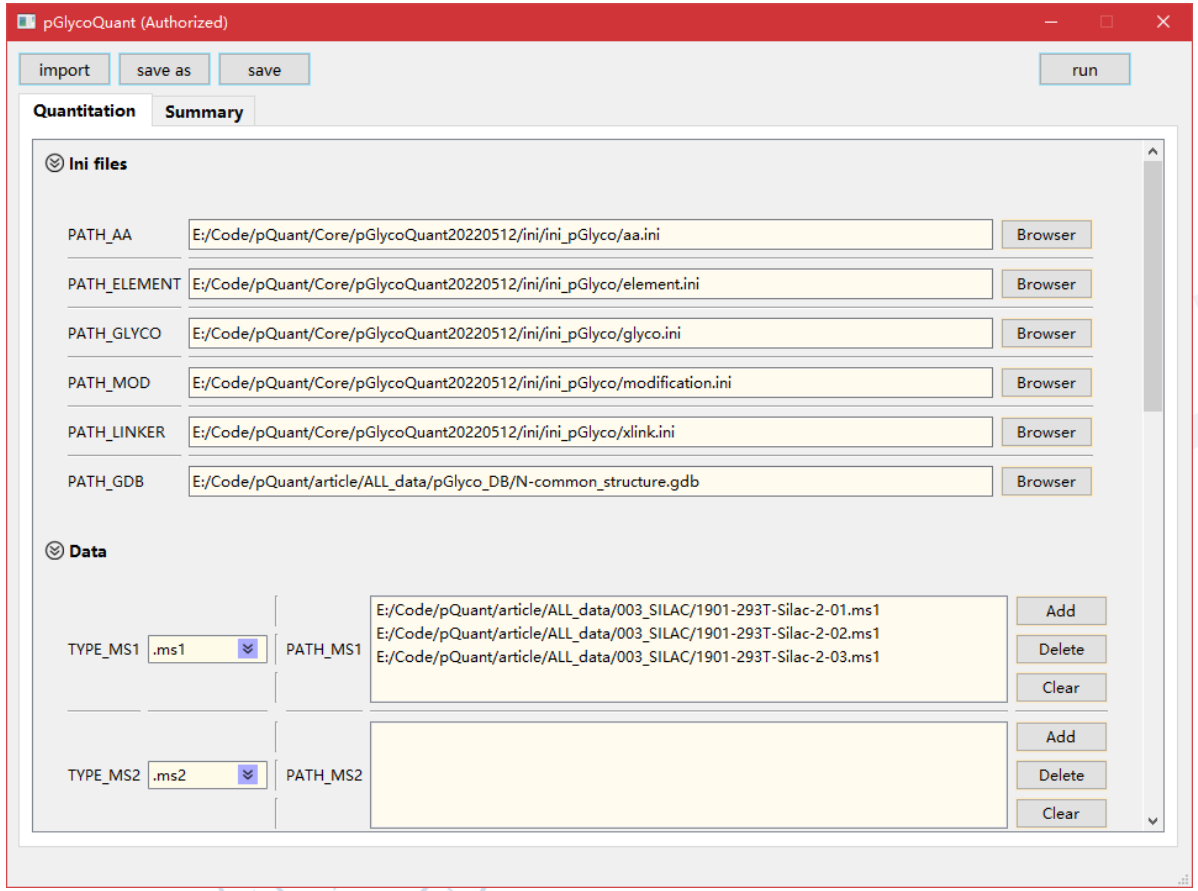
① The completed information in the command-line interface.

② The identification results.

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

SILAC



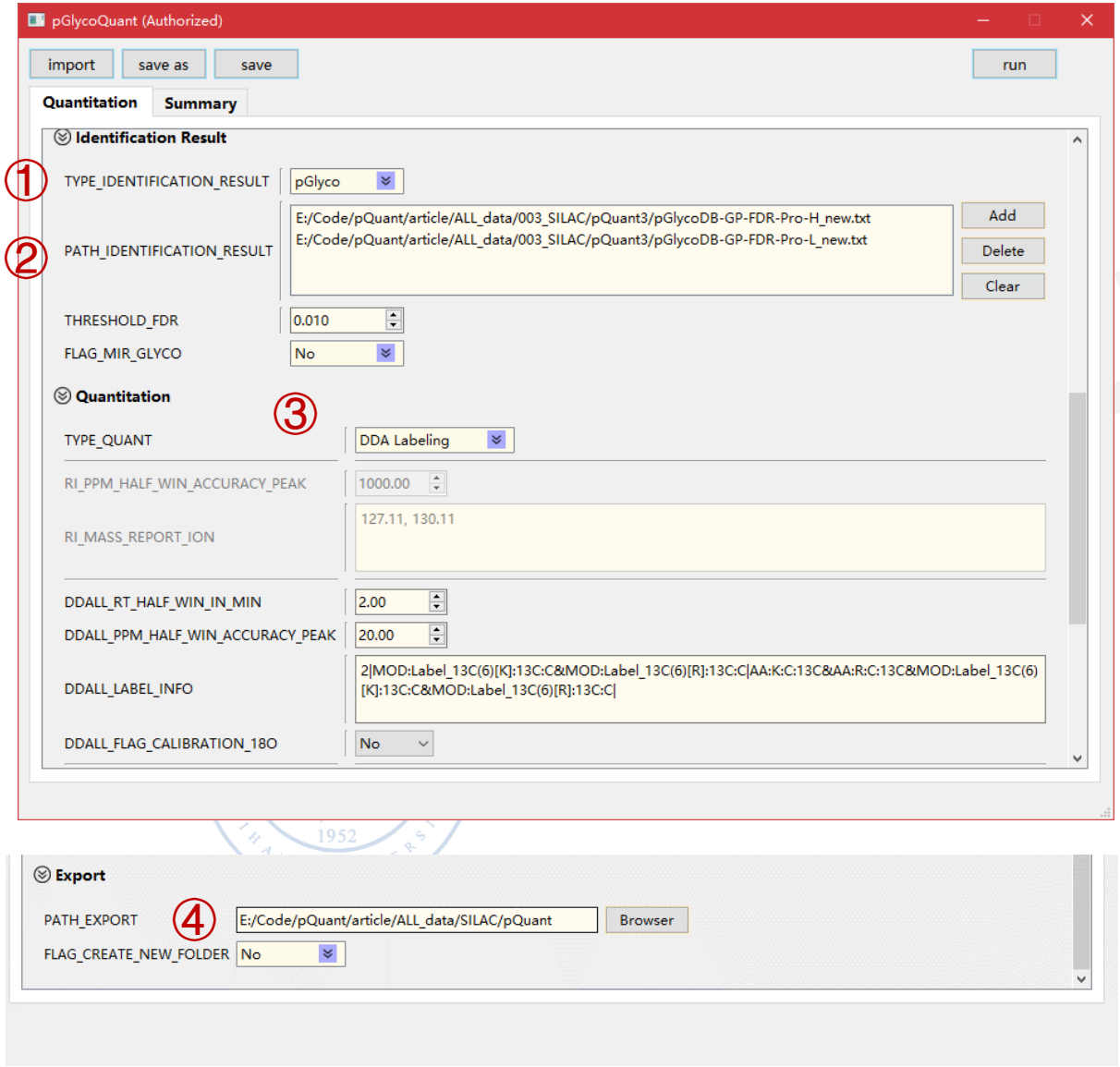
① Ensure that the ini file paths are valid.

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

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

SILAC



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).

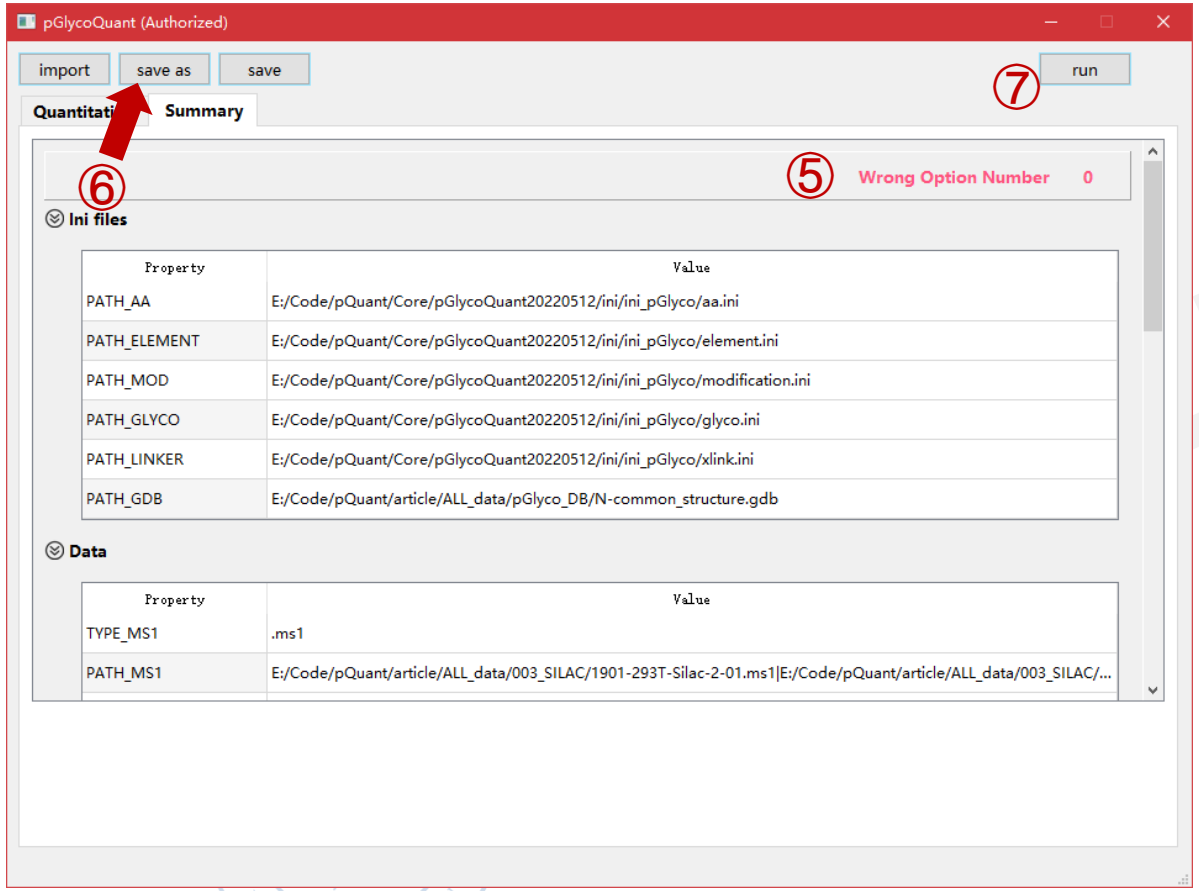
The DDALL_LABEL_INFO could be **2|MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|AA:K:C:13C&AA:R:C:13C&MOD:Label_13C(6)[K]:13C:C&MOD:Label_13C(6)[R]:13C:C|**.

④ Set the Output Folder for saving the quantitation results.

2 Quantitation with pGlycoQuant

1.3 pGlycoQuant quantitation

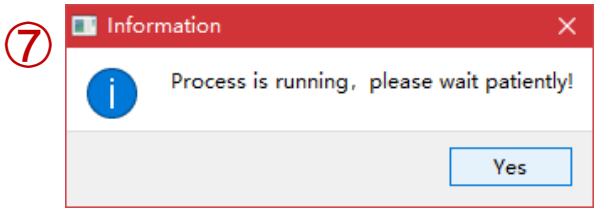
SILAC

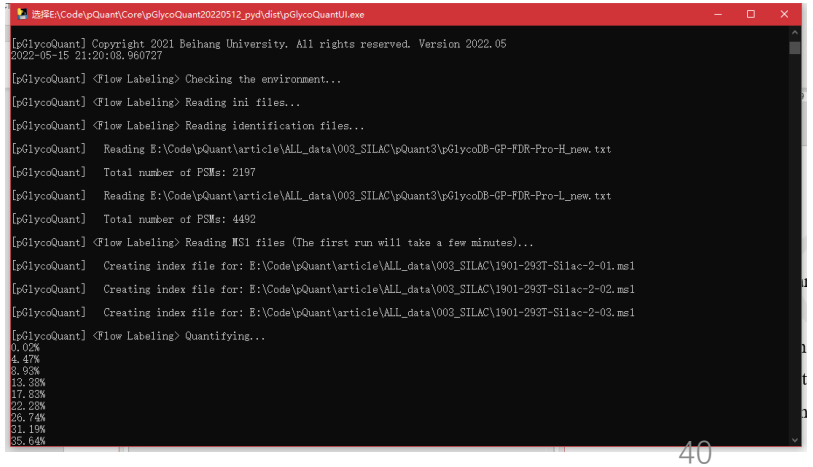


⑤ Click **Summary** button and make sure that the **Wrong Option Number** is **0**.

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

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



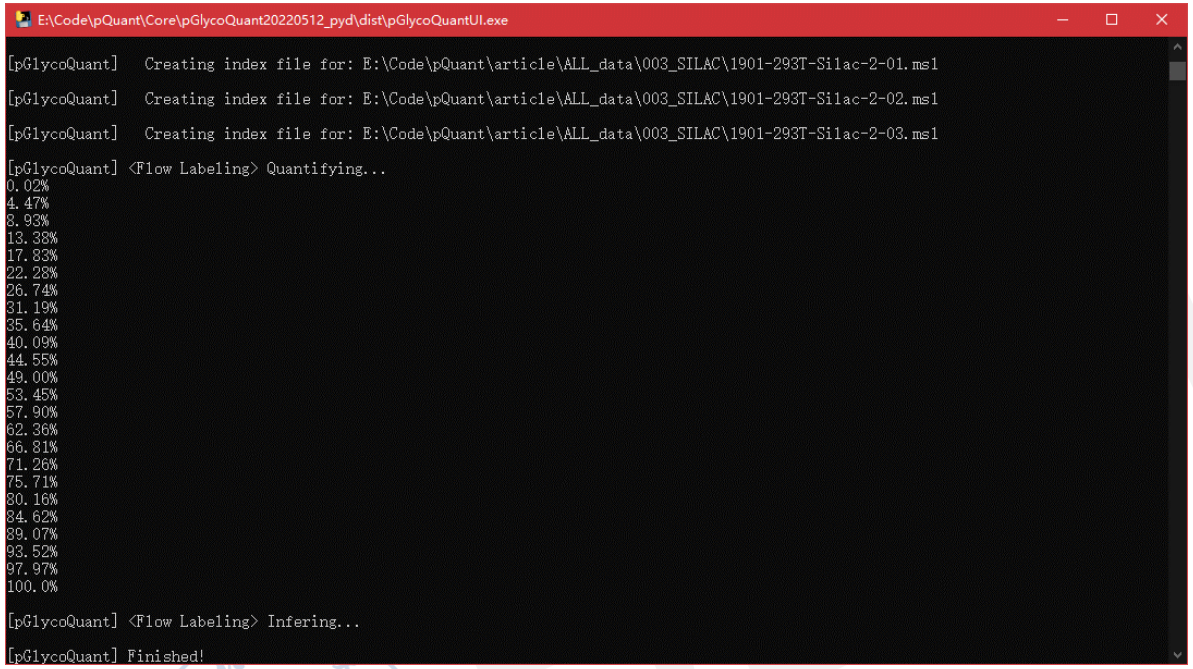


2 Quantitation with pGlycoQuant

1.4 quantitation results

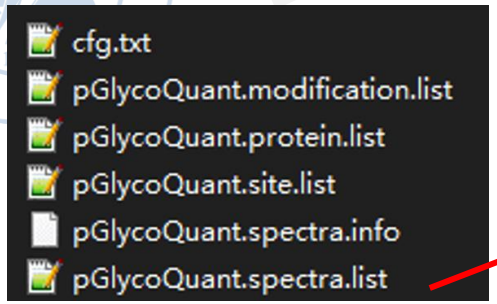
SILAC

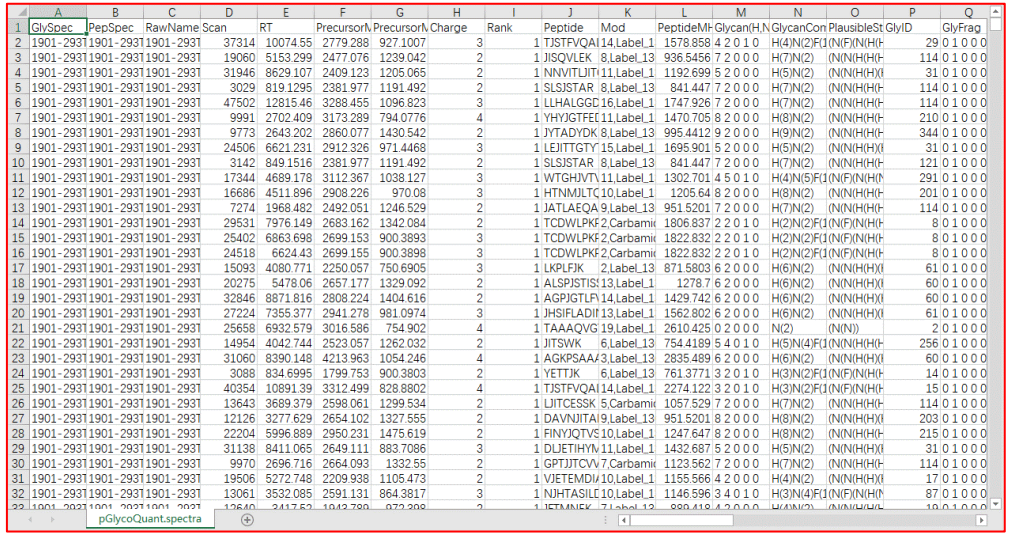
①



② ① The completed information.

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



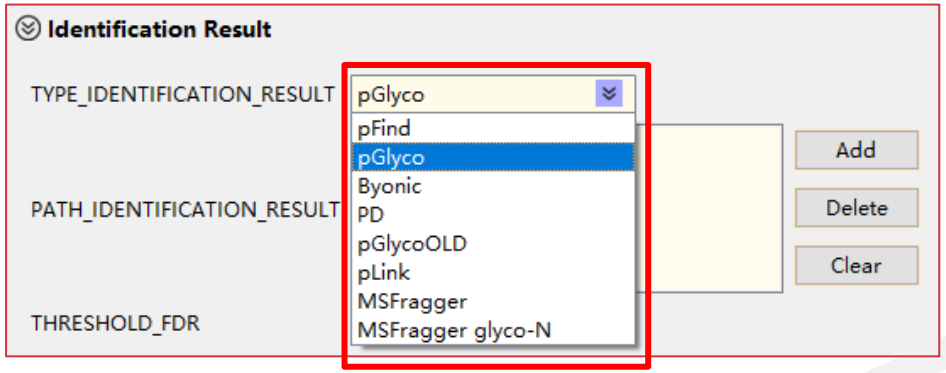


GlySpec	PepSpec	RawName	Scan	RT	Precursor	Precursor	Charge	Rank	Peptide	Mod	PeptideMH	Glycan(H,N	GlycanCon	PlausibleSt	GlyID	GlyFrag
1901-293T	1901-293T	1901-293T	37314	10074.55	2779.288	927.1007	3	1	TJSTFVQAI	14,Label_1	1578.858	4 2 0 1 0	H(4)N(2)F(1	(N(F)(N(H(H	29	0 1 0 0 0
1901-293T	1901-293T	1901-293T	19060	5153.299	2477.076	1239.042	2	1	JISQVLEK	8,Label_13	936.5456	7 2 0 0 0	H(7)N(2)	(N(N(H(H(H-	114	0 1 0 0 0
1901-293T	1901-293T	1901-293T	31946	8629.107	2409.123	1205.065	2	1	NNVITLJIT	11,Label_1	1192.699	5 2 0 0 0	H(5)N(2)	(N(N(H(H)(	31	0 1 0 0 0
1901-293T	1901-293T	1901-293T	3029	819.1295	2381.977	1191.492	2	1	SLSJSTAR	8,Label_13	841.447	7 2 0 0 0	H(7)N(2)	(N(N(H(H(H-	114	0 1 0 0 0
1901-293T	1901-293T	1901-293T	47502	12815.46	3288.455	1096.823	3	1	LLHALGGD	16,Label_1	1747.926	7 2 0 0 0	H(7)N(2)	(N(N(H(H(H-	114	0 1 0 0 0
1901-293T	1901-293T	1901-293T	9991	2702.409	3173.289	794.0776	4	1	YHYJGTFEI	11,Label_1	1470.705	8 2 0 0 0	H(8)N(2)	(N(N(H(H(H-	210	0 1 0 0 0
1901-293T	1901-293T	1901-293T	9773	2643.202	2860.077	1430.542	2	1	JYTADYDK	8,Label_13	995.4412	9 2 0 0 0	H(9)N(2)	(N(N(H(H(H-	344	0 1 0 0 0
1901-293T	1901-293T	1901-293T	24506	6621.231	2912.326	971.4468	3	1	LEJITTGTY	15,Label_1	1695.901	5 2 0 0 0	H(5)N(2)	(N(N(H(H)(	31	0 1 0 0 0
1901-293T	1901-293T	1901-293T	3142	849.1516	2381.977	1191.492	2	1	SLSJSTAR	8,Label_13	841.447	7 2 0 0 0	H(7)N(2)	(N(N(H(H(H-	121	0 1 0 0 0
1901-293T	1901-293T	1901-293T	17344	4689.178	3112.367	1038.127	3	1	WTGHJVT\	11,Label_1	1302.701	4 5 0 1 0	H(4)N(5)F(1	(N(F)(N(H(H(	291	0 1 0 0 0
1901-293T	1901-293T	1901-293T	16686	4511.896	2908.226	970.08	3	1	HTNMJLTC	10,Label_1	1205.64	8 2 0 0 0	H(8)N(2)	(N(N(H(H(H-	201	0 1 0 0 0
1901-293T	1901-293T	1901-293T	7274	1968.482	2492.051	1246.529	2	1	JATLAEQA	9,Label_13	951.5201	7 2 0 0 0	H(7)N(2)	(N(N(H(H(H-	114	0 1 0 0 0
1901-293T	1901-293T	1901-293T	29531	7976.149	2683.162	1342.084	2	1	TCDWLPKF	2,Carbamic	1806.837	2 2 0 1 0	H(2)N(2)F(1	(N(F)(N(H(H-	8	0 1 0 0 0
1901-293T	1901-293T	1901-293T	25402	6863.698	2699.153	900.3893	3	1	TCDWLPKF	2,Carbamic	1822.832	2 2 0 1 0	H(2)N(2)F(1	(N(F)(N(H(H-	8	0 1 0 0 0
1901-293T	1901-293T	1901-293T	24518	6624.43	2699.155	900.3898	3	1	TCDWLPKF	2,Carbamic	1822.832	2 2 0 1 0	H(2)N(2)F(1	(N(F)(N(H(H-	8	0 1 0 0 0
1901-293T	1901-293T	1901-293T	15093	4080.771	2250.057	750.6905	3	1	LKPLFJK	2,Label_13	871.5803	6 2 0 0 0	H(6)N(2)	(N(N(H(H)(	61	0 1 0 0 0
1901-293T	1901-293T	1901-293T	20275	5478.06	2657.177	1329.092	2	1	ALSPJSTIS	13,Label_1	1278.7	6 2 0 0 0	H(6)N(2)	(N(N(H(H)(	60	0 1 0 0 0
1901-293T	1901-293T	1901-293T	32846	8871.816	2808.224	1404.616	2	1	AGPJGTLF\	14,Label_1	1429.742	6 2 0 0 0	H(6)N(2)	(N(N(H(H)(	60	0 1 0 0 0
1901-293T	1901-293T	1901-293T	27224	7355.377	2941.278	981.0974	3	1	JHSIFLADI	13,Label_1	1562.802	6 2 0 0 0	H(6)N(2)	(N(N(H(H)(	61	0 1 0 0 0
1901-293T	1901-293T	1901-293T	25658	6932.579	3016.586	754.902	4	1	TAAAQVG	19,Label_1	2610.425	0 2 0 0 0	N(2)	(N(N))	2	0 1 0 0 0
1901-293T	1901-293T	1901-293T	14954	4042.744	2523.057	1262.032	2	1	JITSWK	6,Label_13	754.4189	5 4 0 1 0	H(5)N(4)F(1	(N(N(H(H(H-	256	0 1 0 0 0
1901-293T	1901-293T	1901-293T	31060	8390.148	4213.963	1054.246	4	1	AGKPSAAJ	3,Label_13	2835.489	6 2 0 0 0	H(6)N(2)	(N(N(H(H)(	60	0 1 0 0 0
1901-293T	1901-293T	1901-293T	3088	834.6995	1799.753	900.3803	2	1	YETTJK	6,Label_13	761.3771	3 2 0 1 0	H(3)N(2)F(1	(N(F)(N(H(H-	14	0 1 0 0 0
1901-293T	1901-293T	1901-293T	40354	10891.39	3312.499	828.8802	4	1	TJSTFVQAI	14,Label_1	2274.122	3 2 0 1 0	H(3)N(2)F(1	(N(F)(N(H(H-	15	0 1 0 0 0
1901-293T	1901-293T	1901-293T	13643	3689.379	2598.061	1299.534	2	1	LJITCESSK	5,Carbamic	1057.529	7 2 0 0 0	H(7)N(2)	(N(N(H(H-	114	0 1 0 0 0
1901-293T	1901-293T	1901-293T	12126	3277.629	2654.102	1327.555	2	1	DAVNJITA	9,Label_13	951.5201	8 2 0 0 0	H(8)N(2)	(N(N(H(H)(	203	0 1 0 0 0
1901-293T	1901-293T	1901-293T	22204	5996.889	2950.231	1475.619	2	1	FINYJQTVS	10,Label_1	1247.647	8 2 0 0 0	H(8)N(2)	(N(N(H(H)(	215	0 1 0 0 0
1901-293T	1901-293T	1901-293T	31138	8411.065	2649.111	883.7086	3	1	DLJETIHYN	11,Label_1	1432.687	5 2 0 0 0	H(5)N(2)	(N(N(H(H)(	31	0 1 0 0 0
1901-293T	1901-293T	1901-293T	9970	2696.716	2664.093	1332.55	2	1	GPTJJTCVV	7,Carbamic	1123.562	7 2 0 0 0	H(7)N(2)	(N(N(H(H-	114	0 1 0 0 0
1901-293T	1901-293T	1901-293T	19506	5272.748	2209.938	1105.473	2	1	VJETEMDI/	10,Label_1	1155.566	4 2 0 0 0	H(4)N(2)	(N(N(H(H)(	17	0 1 0 0 0
1901-293T	1901-293T	1901-293T	13061	3532.085	2591.131	864.3817	3	1	NJHTASILC	10,Label_1	1146.596	3 4 0 1 0	H(3)N(4)F(1	(N(F)(N(H(N	87	0 1 0 0 0

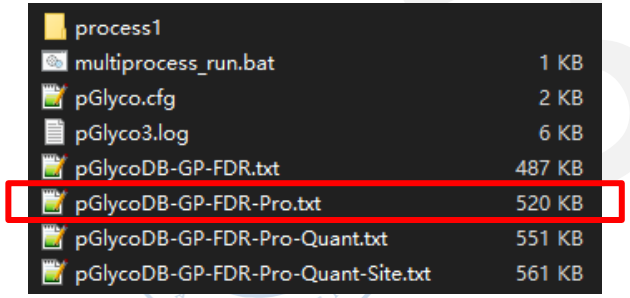
pGlycoQuant.spectra

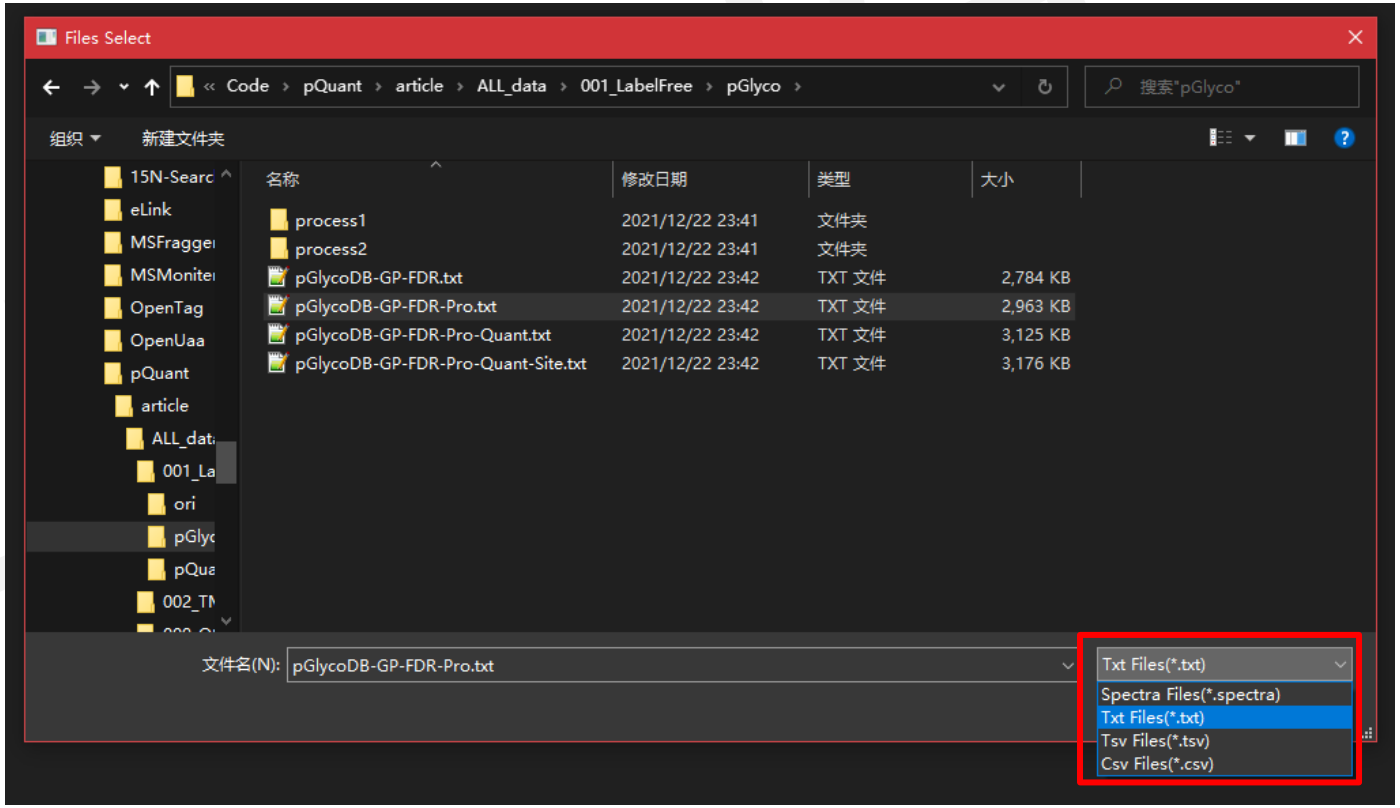
Notes for Choosing the Input File for pGlycoQuant

Choose the related type of identification result for quantitation

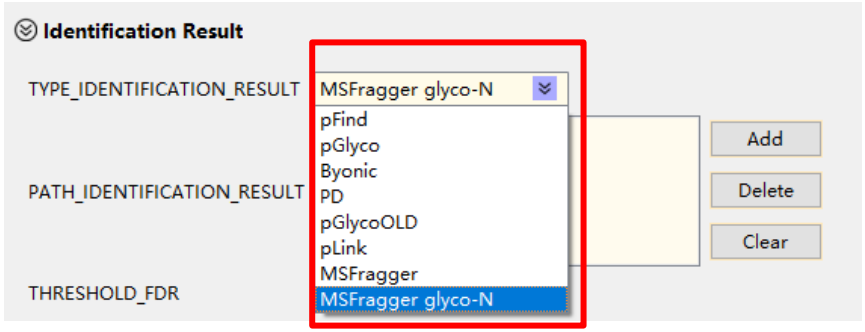


pGlyco results

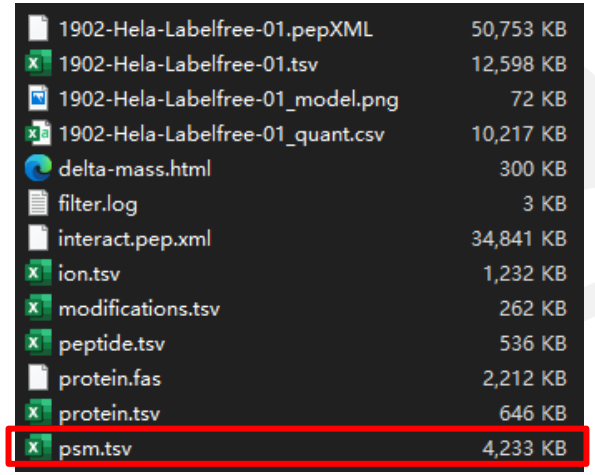


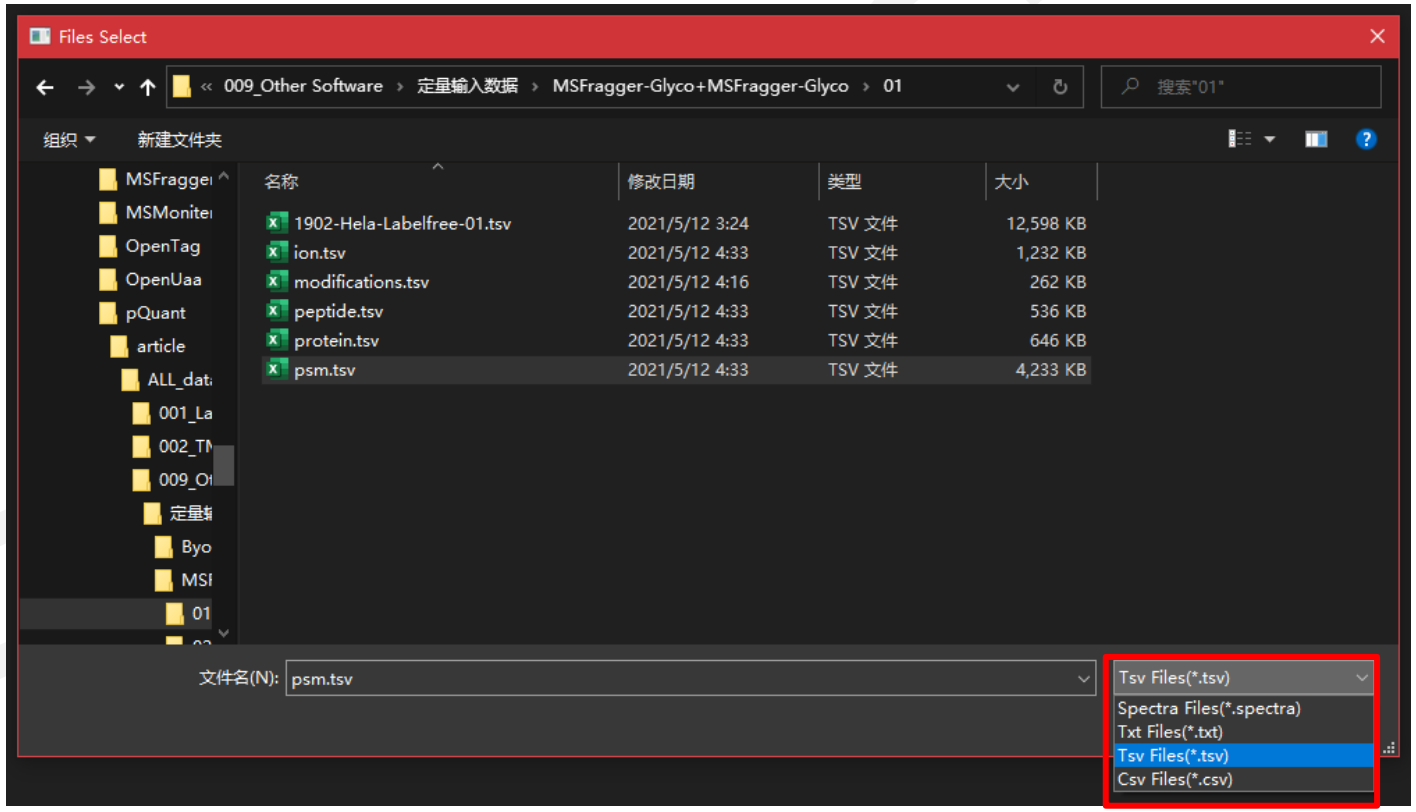


Choose the related type of identification result for quantitation

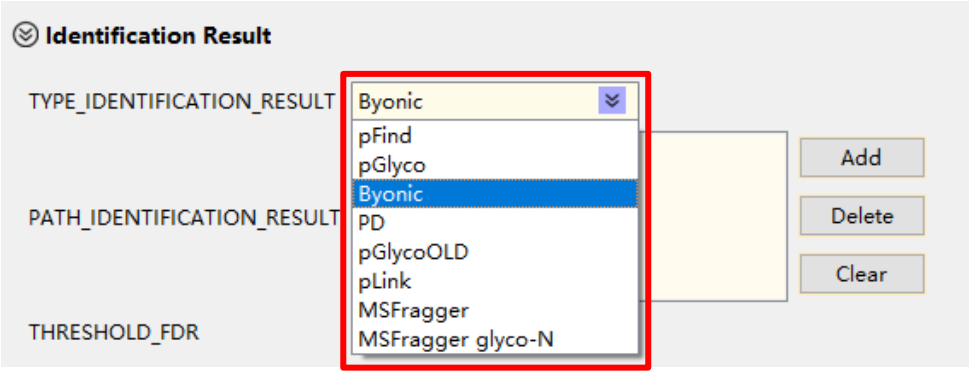


MSFragger results

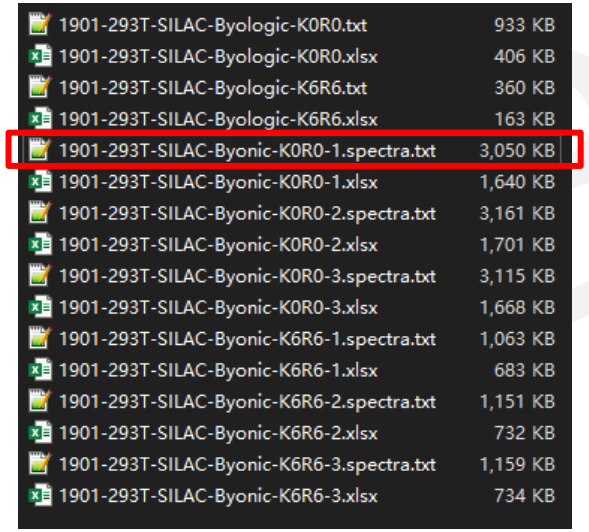


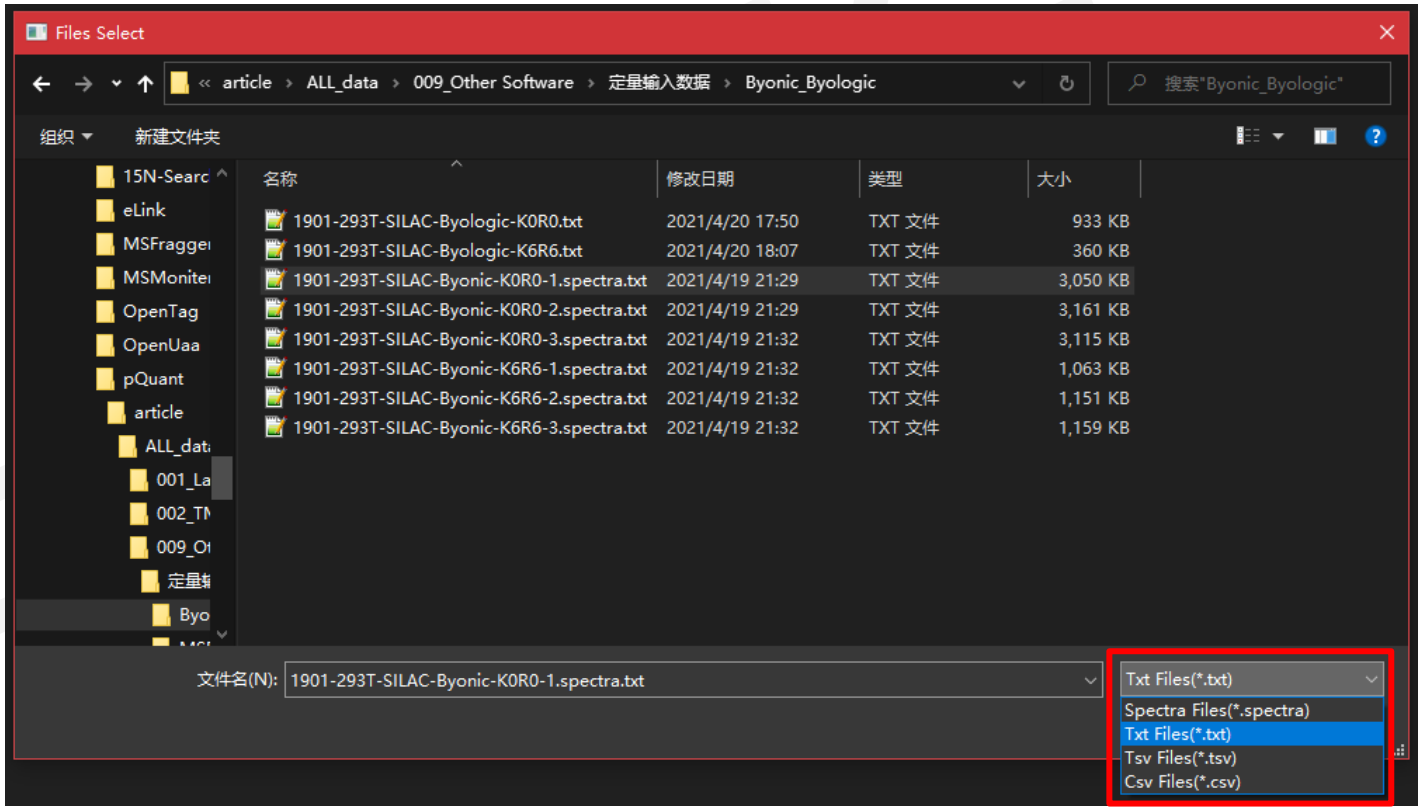


Choose the related type of identification result for quantitation



Byonic results





Thanks!