# Manual of GlycoNote

GlycoNote is a generic and reliable search engine for tandem-MS based glycomics. Here we demonstrate a detailed step-by-step manual for analysis of a typical N-glycomic dataset (step 1~4) and suggested parameters for other distinct glycomic dataset (step 5).

**1 - File description**

There are four different files and a folder in this manual package:

1) “Glyconote-MS2-Identification.exe”:

Executable program of GlycoNote.

2) “Manual-example.mgf”:

MS/MS file for testing. This dataset is a N-glycomic dataset from mouse brain. The RAW data can be found the repository XXX.

3) “Manual-example-parameter.txt”:

Parameter file for testing, detailed description in the next section.

4) “Expected results.zip”:

Expected result files from the testing dataset and parameter.

**2 - Format of parameter file**

The searching parameter is described in detail here. The parameter line is shown in blue; description is shown in black. This parameter is for the analysis of testing MS/MS file (N-glycomic dataset from mouse brain). The parameter templates of other different types of glycomic datasets are shown at the later part of this manual.

*Hex,2,15*

*162.052823*

*HexNAc,2,10*

*203.079374*

*dHex,0,5*

*146.057909*

*NeuAc,0,4,y*

*291.095417*

*NeuGc,0,4,y*

*307.090331*

*Pent,0,0*

*132.042259*

*HexA,0,0*

*176.032081*

Each monosaccharide occupies two lines. The first line represents “name, minimum number, maximum number, require ion”, the second line represents mass. Note that “require ion” (diagnostic peak of this monosaccharide is required for consideration of GSM) is optional and we suggest that this feature should be enabled only on sialic acid.

*SO3,0,0*

*79.956812*

*RED,0,0*

*2.015650*

*ADD,0,0*

*0*

*H2O,1,1*

*18.010563*

Information of other components. The format is the same as above

*# charge\_carrier - Parameter*

*1.007825*

Mass of charge carrier.

*# tolerance\_precursor - Parameter (value, ppm / Da)*

*2,ppm*

Mass tolerance of precursor in the form of “value, unit”.

*# tolerance\_fragment - Parameter (value, ppm / Da)*

*20,ppm*

Mass tolerance of fragment in the form of “value, unit”.

*# c13\_precursor\_analysis - Parameter (yes / no)*

*no*

Additional analysis using the c13 precursor mass.

*# N-glycan filter - Parameter (yes / no)*

*yes*

Filtering of glycan composition using N-glycan biological template.

*# see\_false - Parameter (yes / no)*

*yes*

Output potential fragments that do not match the precursor composition.

*# file\_filter\_diagnostic\_ions - Parameter (yes / no)*

*yes*

Spectral filtering using diagnostic ions.

**3 - How to run the program**

文本

描述已自动生成In a Windows environment, execute “Glyconote-MS2-Identification.exe”. The program will show a command window:

At the same time, the program will ask the user the select MS/MS file and parameter file. Please select “Manual-example.mgf” and “Manual-example-parameter.txt” in the same folder. After the selection of files, the program will perform the analysis and output results automatically, no more human intervention is needed before the program finishes analysis.

文本

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During the running, the command window will present different steps of GlycoNote. Here is a screenshot of ongoing decoy analysis (Supplementary Note 1).

图形用户界面, 文本

描述已自动生成When the analysis finishes, “Spectral analysis finished for file” notice will appear in the command window. At the same time, a popup window showing total running time will appear, as demonstrated by this screenshot.

Two types of result files are generated during the analysis:

1. “Manual-example-Glyconote-Result-MS2.xlsx”， results in table format;
2. Folder of “Manual-example-annotated-spectra”, annotated spectra of all glycan-spectrum matches.

The interpretation of two types of results files is described in the next section.

**4 – Result interpretation**

1. “Manual-example-Glyconote-Result-MS2.xlsx”

There are four different sheets in the Excel file:

“Spectra”:

In the “spectra” sheet, each line is a glycan-spectrum match after FDR filter. The identified glycan composition and scoring are shown.

“Library”

In the “library” sheet, each line is a unique glycan composition with quantitation information in each minute. This sheet provides summarized information of elution windows of each glycan. For example, Fig. 3a in the manuscript is generated using the information in the “library” sheet directly.

“fragments”

In the “fragments” sheet, each annotated peak of each glycan-spectrum match is listed for reference.

“Parameters”

In the “parameters” sheet, the program logs the parameters used in current analysis.

1. Folder of “Manual-example-annotated-spectra

Annotated spectra of all glycan-spectrum matches are saved in the folder. Each glycan-spectrum match is a “png” file. The annotate spectra are used in the manuscript figures and are very useful for manual evaluation.

**5 – Suggested parameters in distinct glycomic dataset**

Suggested parameters for each glycomic dataset presented in the paper are listed and described. Expected results are also included in corresponding folders.

**5.1 - Fig2a-HMO-parameter**

-----Parameter-----

Hex,0,6

162.0528

HexNAc,0,6

203.0794

dHex,0,4

146.0579

NeuAc,0,4,y

291.0954

NeuGc,0,0,y

307.0903

Pent,0,0

132.0423

HexA,0,0

176.0321

HexOAc,0,0

204.0634

SO3,0,0

79.95682

RED,1,1

2.0140

ADD,0,0

0

H2O,1,1

18.0106

# charge\_carrier - Parameter

1.0073

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

yes

# N-glycan filter - Parameter (yes / no)

no

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

NeuGc,0,0,y

307.0903

No NeuGc in this sample.

RED,1,1

2.0140

Reduction was performed on this sample.

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

Mass accuracy of the QTOF instrument used.

# N-glycan filter - Parameter (yes / no)

no

The sample is not a N-glycomics sample.

**5.2 - Fig2b-O-Glycome-Human-parameter**

-----Parameter-----

Hex,0,4

162.0528

HexNAc,0,4

203.0794

dHex,0,4

146.0579

NeuAc,0,4,y

291.0954

NeuGc,0,4,y

307.0903

Pent,0,0

132.0423

HexA,0,0

176.0321

HexOAc,0,0

204.0634

SO3,0,0

79.95682

RED,1,1

2.0140

ADD,0,0

0

H2O,1,1

18.0106

# charge\_carrier - Parameter

1.0073

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

yes

# N-glycan filter - Parameter (yes / no)

no

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

RED,1,1

2.0140

Reduction was performed on this sample.

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

Mass accuracy of the QTOF instrument used.

# N-glycan filter - Parameter (yes / no)

no

The sample is not a N-glycomics sample.

**5.3 - Fig2c-N-Glycome-Human-parameter**

-----Parameter-----

Hex,3,12

162.0528

HexNAc,2,7

203.0794

dHex,0,4

146.0579

NeuAc,0,4,y

291.0954

NeuGc,0,4,y

307.0903

Pent,0,0

132.0423

HexA,0,0

176.0321

HexOAc,0,0

204.0634

SO3,0,0

79.95682

RED,0,0

2.0140

ADD,0,0

0

H2O,1,1

18.0106

# charge\_carrier - Parameter

1.0073

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

yes

# N-glycan filter - Parameter (yes / no)

yes

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

Mass accuracy of the QTOF instrument used.

**5.4 - Fig2d-Poly-Plant-parameter**

-----Parameter-----

Hex,0,6

162.0528

HexNAc,0,3

203.0794

dHex,0,3

146.0579

NeuAc,0,0,y

291.0954

NeuGc,0,0,y

307.0903

Pent,0,2

132.0423

HexA,0,2

176.0321

HexOAc,0,0

204.0634

SO3,0,0

79.95682

RED,1,1

2.0140

ADD,0,0

0

H2O,1,1

18.0106

# charge\_carrier - Parameter

1.0073

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

yes

# N-glycan filter - Parameter (yes / no)

no

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

NeuAc,0,0,y

291.0954

NeuGc,0,0,y

307.0903

Pent,0,2

132.0423

HexA,0,2

176.0321

No Sialic acids in this sample. Pent and HexA are presented in this sample.

RED,1,1

2.0140

Reduction was performed on this sample.

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

Mass accuracy of the QTOF instrument used.

# N-glycan filter - Parameter (yes / no)

no

The sample is not a N-glycomics sample.

**5.5 - Fig2ef-N-Glycome-C.elegans-parameter**

-----Parameter-----

Hex,2,15

162.052823

HexNAc,2,10

203.079374

dHex,0,5

146.057909

NeuAc,0,4,y

291.095417

NeuGc,0,4,y

307.090331

Pent,0,0

132.042259

HexA,0,0

176.032081

SO3,0,0

79.956812

RED,0,0

2.015650

ADD,0,0

0

H2O,1,1

18.010563

# charge\_carrier - Parameter

1.007825

# tolerance\_precursor - Parameter (value, ppm / Da)

2,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

20,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

no

# N-glycan filter - Parameter (yes / no)

no

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

# N-glycan filter - Parameter (yes / no)

no

The sample includes atypical N-glycans, so this parameter is set to “no”.

**5.6 - Fig3a-N-Glycome-Human-parameter**

-----Parameter-----

Hex,2,15

162.052823

HexNAc,2,10

203.079374

dHex,0,5

146.057909

NeuAc,0,4,y

291.095417

NeuAcL,0,4,y

366.142686

NeuGc,0,0,y

307.090331

Pent,0,0

132.042259

HexA,0,0

176.032081

SO3,0,0

79.956812

RED,0,0

2.015650

ADD,0,0

0

H2O,1,1

18.010563

# charge\_carrier - Parameter

1.007825

# tolerance\_precursor - Parameter (value, ppm / Da)

5,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

20,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

no

# N-glycan filter - Parameter (yes / no)

yes

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

NeuAcL,0,4,y

366.142686

Labelled NeuAc in this sample.

**5.7 - Fig3b-N-Glycome-Mouse-parameter**

-----Parameter-----

Hex,2,15

204.0998

HexNAc,2,10

245.1263

dHex,0,5

174.0892

NeuAc,0,4,y

361.1737

NeuGc,0,4,y

391.1842

Pent,0,0

160.0736

HexA,0,0

218.079

SO3,0,0

79.956812

RED,0,0

2.015650

ADD,0,0

0

H2O,1,1

18.010563

# charge\_carrier - Parameter

1.007825

# tolerance\_precursor - Parameter (value, ppm / Da)

2,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

20,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

no

# N-glycan filter - Parameter (yes / no)

yes

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

Hex,2,15

204.0998

HexNAc,2,10

245.1263

dHex,0,5

174.0892

NeuAc,0,4,y

361.1737

NeuGc,0,4,y

391.1842

Pent,0,0

160.0736

HexA,0,0

218.079

Permethylated mass of each monosaccharide is used.

**5.8 - Fig3c-O-Glycome-Human-parameter**

-----Parameter-----

Hex,0,4

162.0528

HexNAc,0,4

203.0794

dHex,0,4

146.0579

NeuAc,0,4,y

291.0954

NeuGc,0,4,y

307.0903

SiaNAz,0,4,y

332.0963

Pent,0,0

132.0423

HexA,0,0

176.0321

HexOAc,0,0

204.0634

SO3,0,0

79.95682

RED,1,1

2.0140

ADD,0,0

0

H2O,1,1

18.0106

# charge\_carrier - Parameter

1.0073

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

yes

# N-glycan filter - Parameter (yes / no)

no

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

SiaNAz,0,4,y

332.0963

Labelled NeuAc in this sample.

RED,1,1

2.0140

Reduction was performed on this sample.

# tolerance\_precursor - Parameter (value, ppm / Da)

20,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

100,ppm

Mass accuracy of the QTOF instrument used.

# N-glycan filter - Parameter (yes / no)

no

The sample is not a N-glycomics sample.

**5.9 - Fig4b-N-Glycome-Mouse-parameter**

-----Parameter-----

Hex,2,15

162.052823

HexNAc,2,10

203.079374

dHex,0,5

146.057909

NeuAc,0,4,y

291.095417

NeuGc,0,4,y

307.090331

Pent,0,0

132.042259

HexA,0,0

176.032081

SO3,0,0

79.956812

RED,0,0

2.015650

ADD,0,0

0

H2O,1,1

18.010563

# charge\_carrier - Parameter

1.007825

# tolerance\_precursor - Parameter (value, ppm / Da)

2,ppm

# tolerance\_fragment - Parameter (value, ppm / Da)

20,ppm

# c13\_precursor\_analysis - Parameter (yes / no)

no

# N-glycan filter - Parameter (yes / no)

yes

# see\_false - Parameter (yes / no)

yes

# file\_filter\_diagnostic\_ions - Parameter (yes / no)

yes

-----Description of changes, compared with the manual example parameters-----

(Same parameters used as that of the example dataset)