

A Simple User-Guide of pGlycoNovo

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A simple GUI guide

pGlyco3

Raw Protein Glycan Search

Load Parameters

Raw files

File Type: raw Fragmentation: HCD

File Name	Size
C:\DataSets\pGlycoTest\MouseBrain\Model-Mouse-Brain-H-Try-4.raw	2.40 GB

Add

Remove

Clear All

In the “Raw” tab, click “Add” to add raw files

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Raw Protein Glycan Search

Fasta:

Enzyme

Name: Digest N-Term: Digest C-Term: Max Miss Cleavage:

Digestion:

Fixed Protein Modifications

Variable Protein Modifications

Modification List

- 2-dimethylsuccinyl[C]
- 2-monomethylsuccinyl[C]
- 2-nitrobenzyl[Y]
- 2-succinyl[C]
- 2HPG[R]
- 3-deoxyglucosone[R]
- 3-phosphoglyceryl[K]
- 3sulfo[AnyN-term]
- 4-ONE+Delta_H(-2)O(-1)[C]
- 4-ONE+Delta_H(-2)O(-1)[H]
- 4-ONE+Delta_H(-2)O(-1)[K]
- 4-ONE[C]
- 4-ONE[H]
- 4-ONE[K]
- 4AcAllylGal[C]
- ADP-Ribosyl[C]
- ADP-Ribosyl[D]
- ADP-Ribosyl[E]
- ADP-Ribosyl[K]

Filter:

Max Var Mod on Peptide: Peptide Length: from to Peptide Mass: from to

In the “Protein” tab, click
“Browse” to add a fasta file.

Modifications and other protein-
level settings are also configured
here.

pGlyco3


Raw Protein Glycan Search

Glycan DB: Glycan Type:

Fixed Glycan Modifications

Variable Glycan Modifications

Max Var Mod on Glycan: Max Number of (Modified) Glycans to Search:



“Glycan” tab is not required for pGlycoNovo search

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Raw Protein Glycan Search

Precursor Tolerance: \pm 10 ppm Fragment Tolerance: \pm 20 ppm

Number of Processes: 5 Glycopeptide FDR: 0.01

☐ Percolator ☐ FMM for Peptide FDR

☒ pGlycoSite: Localized Glycans Must Be in GDB

pGlycoNovo

☒ Run pGlycoNovo

Glyco:	H	Max:	20
Glyco:	N	Max:	7
Glyco:	F	Max:	5
Glyco:	A	Max:	4
Glyco:		Max:	
Glyco:		Max:	
Glyco:		Max:	
Glyco:		Max:	

Allow Max Glyco Gap: 3

Output Folder: Browse

Run Save Stop

In “Search” tab, check “Run pGlycoNovo”:

Set the “Glyco” type and its “Max” number;

Set “Allow Max Glyco Gap” as 2 (accurate but less sensitive) or 3 (median accurate and sensitive) or 4 (less accurate but more sensitive)

Browse “Output Folder” to select output folder.

Click “Run” to search

Result interpretation

Title interpretation of the report table (e.g., pGlycoNovo-GP-FDR.txt)

GlySpec	Spectrum name
PepSpec	Spectrum name
RawName	Raw name
Scan	Scan num
RT	Retention time in seconds
PrecursorMH	Precursor Mass + H
PrecursorMZ	Precursor m/z
Charge	Charge state
Rank	Rank of the GPSM, always 1
Peptide	Peptide sequence
Mod	Modifications
PeptideMH	Peptide Mass + H
Glycan(H,N,F,A,X)	Glycan composition in vector format (the order of glyco)
GlycanComposition	Glycan composition in string format
GlyFrag	Glycan fragments
GlyMass	Glycan mass
GlySite	Glycan site (usually accurate for N-glycan, but not accurate for O-glycan)

Title interpretation of the report table (e.g., pGlycoNovo-GP-FDR.txt)

OriginTotalScore	Weighted sum of original glycan score and peptide score, before percolator rescoring
OriginPepScore	Peptide score before percolator
OriginGlyScore	Glycan score before percolator
CoreMatched	Number of matched glycan
MassDeviation	Mass deviation in Da
PPM	Mass deviation in PPM
GlyDecoy	Is glycan decoy? 1 yes, 0 no
PepDecoy	Is peptide decoy? 1 yes, 0 no
GlyScore	Glycan score for FDR
PepScore	Peptide score for FDR, same as OriginPepScore if percolator is not applied
TotalScore	Combination of GlyScore and PepScore
IsSmallGlycan	Number of glycos <= 3
GlycanPEP	Always 0 for pGlycoNovo
GlycanFDR	Always 0 for pGlycoNovo as no FDR methods on glycan
PeptidePEP	Peptide posterior error probability, not -1 only when FMM is applied to estimate FDR
PeptideFDR	Peptide FDR (Q-value)
TotalFDR	Same as PeptideFDR for pGlycoNovo