MaxQuant.Live Documentation

Release stable

Dec 10, 2019

Overview

This is the user documentation of MaxQuant.Live, a software framework for real-time monitoring of mass spectrometric data and controlling of the data acquisition. MaxQuant.Live can be downloaded on http://maxquant.live

Publication: MaxQuant.Live enables global targeting of more than 25,000 peptides

Christoph Wichmann, Florian Meier, Sebastian Virreira Winter, Andreas-David Brunner, Juergen Cox, Matthias Mann doi: 1101/443838

Note: This user documentation is not fully complete yet and is frequently updated.

User Interface

The main window of MaxQuant.Live consists of three sections, which you can open by clicking on the symbols in the main menu.

1.1 Instrument

The instrument type can be selected here connected using the "connect instrument" button. In this ver-sion, only Thermo Fisher Scientific Q Exactive HF-X mass spectrometers are supported (Tune Version 2.9).

1.2 Main Module

This part of the software is the link between the instrument and the scan protocol Library. It consists of a log message window and the configuration of the log directory. It is recommended to always load the log directory before connecting the instrument. The files written to this directory contain all log messages that were also shown in the graphical user interface. There are two types of log file written out by MaxQuant.Live Global log file: MaxQuant.Live will create global log files on every day. The filename is the date itself. These files contain all the log messages that were thrown when no scan protocol was running. Specific log files: During the execution of a scan protocol, the log messages are written to scan proto-col specific log file. The file name starts with the four-digit identification number, followed by the date and the time of execution. When the scan protocol ended, the log messages are again written into the global log file.

1.3 Scan Protocol Library

Scan protocols implement various the acquisition strategies supported by our software. Every scan proto-col has a certain type (BoxCar, EasiTag...), a unique four-digit identification number and a short experiment description. All scan protocols are stored in a library. In the scan protocol section of the software you can manage the library.
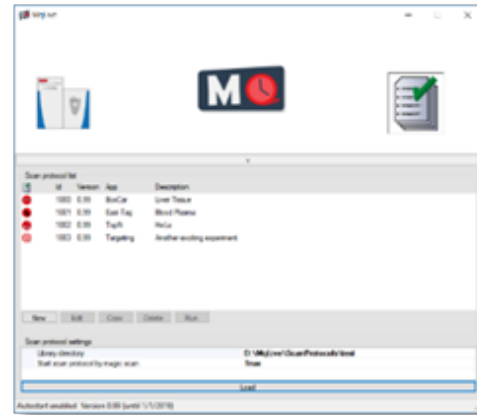
Operation Modes

The logo in the middle of the symbol bar of MaxQuant.Live displays the current operation mode of the software. There are three operation modes:

2.1 Setup Mode

This is the initial operation mode after software startup. In this modus, three parts of MaxQuant.Live (Sec. 4) can be configured. After loading the log-directory, the scan protocol library and connecting the instru-ment, MaxQuant.Live switches to the Listening Mode.
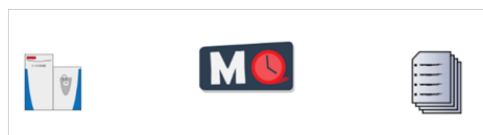
2.2 Listening Mode

In this mode, the software receives all the spectra acquired by the instrument but does not send any commands to the mass spectrometer. MaxQuant.Live is therefore not affecting the data acquisition of the mass spectrometer. Scan protocols can be started in the software is in this operation mode. After the startup of a scan protocol, MaxQuant.Live switches to the Running Mode.

2.3 Running Mode

This operation mode is active during the execution of a scan pro-tocol. Afterwards, the software automati-cally switches back to the listening mode.

Installation
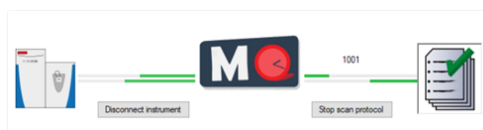
3.1 Check requirements

- Microsoft .NET Framework 4.7.2
- Q-Exactive Tune 2.9
- No IAPI licence is required
- Thermo Fisher Q-Exactive Mass Spectrometer HF/HF-X

Note: Q Exactive classic/plus instruments were not tested and are not officially supported.

3.2 Download and unpack

The latest version 0.99 can be downloaded from http://maxquant.live and is valid until 1/1/2019. Unpack the zip file in a new folder on the local hard drive of the acquisition computer.
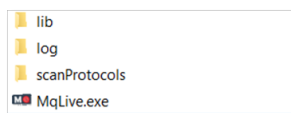
If necessary, create empty 'log' and 'scanProtocols' folders manually. To set up MaxQuant.Live, continue reading here.

Create and edit Scan Protocols

4.1 New scan protocol

When you click on the "New" button, the app store opens. First, select the acqui-sition method that should be used in the new scan protocol. Click on the big symbol to open the settings for the selected app. A green checkmark indicates which app has been selected. Next, select a unique identification number for your scan protocol. The list in the drop-down menu excludes id numbers that are already present in the library. Finally fill in a short description of the experiment and click create. The scan protocol will be saved as file in the scan protocol folder and appear in the scan protocol library.

4.2 Edit scan protocols

Scan protocols in the library can be edited later by clicking the "Edit" button. It is possible to change the global properties like the ID number and the short description of the scan protocol. Furthermore, it is possible to edit the specific parameters of an acquisition strategy using the respective app.

4.3 Copy and Delete

Existing scan protocols can be easily copied by clicking the "Copy" button. The copy gets automatically an ID number assigned, is added to the library and can be edited afterwards by the user. Existing Scan Protocols can also be deleted by the user by clicking the "Delete" button.

4.4 Storage and Compatibility

Scan protocols are saved as files ("mqlive") local library, which serves as scan protocol library. The path has to be set at when the software is started but can be changed after-wards during runtime. Therefore is it possible to use several scan protocol libraries for different experi-ments or users. Please note that every scan protocol has a version number, which is the version of the MaxQuant.Live that has been used to create or edit it the last time. Compatibility is not guaranteed if the scan protocol the MaxQuant.Live version differ. In this case, edit the settings to check the parameters and save the scan protocol to update the version number.

4.5 Run scan protocol

Continue reading here

Run Scan protocols

When MaxQuant.Live is in Listening Mode scan protocols can be started either manually or in an automated manner.

5.1 Manual startup

To be able to start a scan protocol manually, the instrument has to be in Running Mode. Therefore, is has to run idle or an Xcalibur method. Check, by using the control software Tune, if the instrument is constantly performing scans. Only in this instrument modus can MaxQuant.Live start scan protocols. To start up a scan protocol, navigate to the library, select the desired scan protocol and click the "run" button. MaxQuant.Live switches into the Running Mode. While a scan protocol is running, you can see its Id number in the symbol bar right of the GUI. MaxQuant.Live is now directing the data acquisition according to the logic implemented in the scan protocol. If you switch to Tune, then you should see incoming log messages indicating that MaxQuant.Live took over the control of the mass spectrometer. Switching back to MaxQuant.Live, the execution of the scan protocol can be terminated by clicking the "Stop scan protocol" button. MaxQuant.Live then switches back into Listening Mode.

5.2 Automated startup

The startup of a scan protocol can be triggered by an incoming so-called "magic scan". If MaxQuant.Live is in Listening Mode and receives Ms1 scans with the scan range m/z 909 to XXXX, then the scan protocol with Id number XXXX is loaded from the library and started immediately. This feature enables a seamless integration of MaxQuant.Live into the standard LC-MS controlled by Xcalibur, because the magic scans can be easily defined as Xcalibur method. Doing this, MaxQuant.Live switches into Running Mode and takes over the control directly after a 'magic' Xcalibur method starts.

5.2.1 Xcalibur method setup

1. Create a new Xcalibur method via the standard method editor. Enter LC settings, Tune files, etc. as you would normally do. Please refer to the Xcalibur user manual for help. Caution: Make sure that the MS method duration is equal to your LC run time.

2. In the MS method, create one Full MS – SIM scan with the scan range m/z 909 to MaxQuant.Live Id, for example m/z 909 to 1001 for the MaxQuant.Live scan protocol with the unique identifier 1001 created above. Set the scan runtime to an arbitrary value >1 min, but less than the method duration. The scan resolution should be equal to the Full Scan resolution set in the MaxQuant.Live scan protocol.

3. Add a second Full MS – SIM scan as in Step 2, but now set the scan range from m/z 908 to "1000 + LC run time", for example m/z 908 to 1100 for a 100 min LC-MS experiment. This step is required! If you forget it your instrument can switch to mode in which MaxQuant.Live won't start anymore. In this case the instrument has to be restarted.

4. Review all settings and save the method.

5.2.2 Data acquisition

1. Make sure that MaxQuant.Live is in Listening mode.

2. Check if your scan protocol library is loaded and the scan protocol with ID number defined before (1001) is present in the list. The setting "Start scan protocol by magic scan" has to be true (default).

3. Open the Xcalibur software and set up a new acquisition sequence. Select the Xcalibur method generated above as the instrument method for your BoxCar runs. Note that different MaxQuant.Live protocols require different Xcalibur methods.

4. Start the acquisition sequence. MaxQuant.Live will take over control as soon as the trigger signal (full MS scan with a scan range starting at m/z 909, 'magic scan') is identified. The home view will indicate that the software is now in Running Mode (Sec. 5.3). Acquisition will stop automatically at the end of the LC-MS run and the Xcalibur sequence will continue with the next run (if applicable). Stop scan protocol stops any running protocol immediately.

	Sample Type	Position	Inj Vol	File Name	Path	Inst Meth
1	Unknown	A1	1.0	Raw_file_name	D:\	C:\Xcalibur\methods\ . . . . 100min_id1001
*			0.0			

5. Disconnect the instrument and close MaxQuant.Live once all experiments are finished.
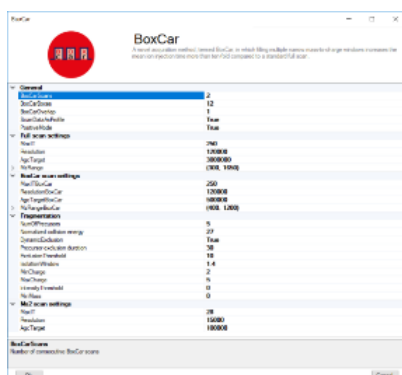
BoxCar

BoxCar acquisition method enables single-shot proteomics at a depth of 10,000 proteins in 100 minutes *Florian Meier, Philipp E. Geyer, Sebastian Virreira Winter, Juergen Cox & Matthias Mann*

The BoxCar acquisition method for high resolution mass spectrometry-based proteomic builds on the combined acquisition of narrow m/z windows to enhance the mean ion filling time as compared to stand-ard full scans.

6.1 Acquisition

All MS parameters are defined and saved in MaxQuant.Live scan protocols. The pop-up window allows you to set all instrument parameters required for BoxCar runs. The acquisition cycle comprises one Full Scan, N BoxCar scans and Z data-dependent MS2 scans. Precursors for MS2 are se-lected from the Full Scan. Note that the mass resolution in the full scan must equal the mass resolution setting in the BoxCar scans for MaxQuant data analysis. For BoxCar scans, each box will be assigned a maximum ion injection time of 1/N th of the total, for example 10 ms for each of 10 boxes per scan with a total max. ion injection time of 100 ms. The same applies to the AGC target value. The BoxCar window placement will be automatically scaled to the expected m/z distribution of tryptic peptides.

Warning: Choosing a too high AGC target for BoxCar scans may cause space-charge effects by overloading the Orbitrap.

**Warning:** Currently, only profile scans are recognized by MaxQuant.

Method parameter	Description
General	
BoxCar Scans	Number of BoxCar scans per acquisition cycle
BoxCar Boxes	Number of boxes per BoxCar scan
BoxCar overlap	M/z overlap of neighboring boxes (Th)
ScanDataAsProfile	Profile (true) or centroid (false) spectra
Positive mode	Electrospray polarity
Full Scan settings	
MaxIT	Maximum ion injection time (ms)
Resolution	MS resolving power at m/z 200
AGCtarget	AGC target value (charges)
MzRange	Scan range (m/z)
BoxCar scan settings	
MaxITBoxCar	Maximum total ion injection time (ms) for BoxCar scans. Each box is assigned 1/N th of this value.
ResolutionBoxCar	MS resolving power at m/z 200
AgcTargetBoxCar	Total AGC target value for each BoxCar scan (charges). Each box is assigned 1/N th of this value.
MzRangeBoxCar	Scan range (m/z)
Fragmentation	
NumOfPrecursors	Number of precursor ions per acquisition cycle
Normalized collision energy	Normalized collision energy (NCE)
DynamicExclusion	Dynamic exclusion time of precursors after first spectrum (s)
Exclusion mass tolerance	Mass tolerance for dynamic exclusion (ppm)
Exclusion threshold	Intensity threshold for dynamic exclusion
Isolation window	Width of the precursor isolation window (Th)
MinCharge	Minimum charge state of the precursor
MaxCharge	Maximum charge state of the precursor
Intensity threshold	Precursor intensity threshold (cps)
MinMass	Minimum mass threshold for precursor ions (Da)
Ms2 scan settings	
MaxIT	Maximum ion injection time (ms)
Resolution	MS resolving power at m/z 200
AgcTarget	AGC target value (charges)

6.2 Analysis

MaxQuant is a software package for quantitative high resolution mass-spectrometry based proteomics (Cox and Mann, Nature Biotechnology 2008 (26), 1367-1372, doi: 10.1038/nbt.1511) and can be downloaded from http://www.maxquant.org. We have recently published a detailed and up-to-date description of the MaxQuant computational platform (Tyanova et al., Nature Protocols 2016 (11), 2301–2319, doi: 10.1038/nprot.2016.136). Please refer to this publication for information about different processing workflows and a detailed description of the parameters. Here, we provide a step-by-step protocol to analyze raw files acquired with the BoxCar method. Default parameters were used unless indicated.

1. Open a new MaxQuant (v1.6.1.0 or later) session and load or 'drag and drop' Thermo .raw files into the MaxQuant window.

2. Set experiment name for each raw file (or load experimental design template if applicable).

3. Set parameter groups for each raw file. BoxCar and shotgun experiments need to be in separate parameter groups. Fractions (if applicable) should not be specified when matching from a fractionated peptide library into single runs is intended.

4. Set group specific parameters. Select "Type / Standard" for each experiment group containing regular shotgun experiments and "Type / BoxCar" for each group containing BoxCar experiments, respectively.
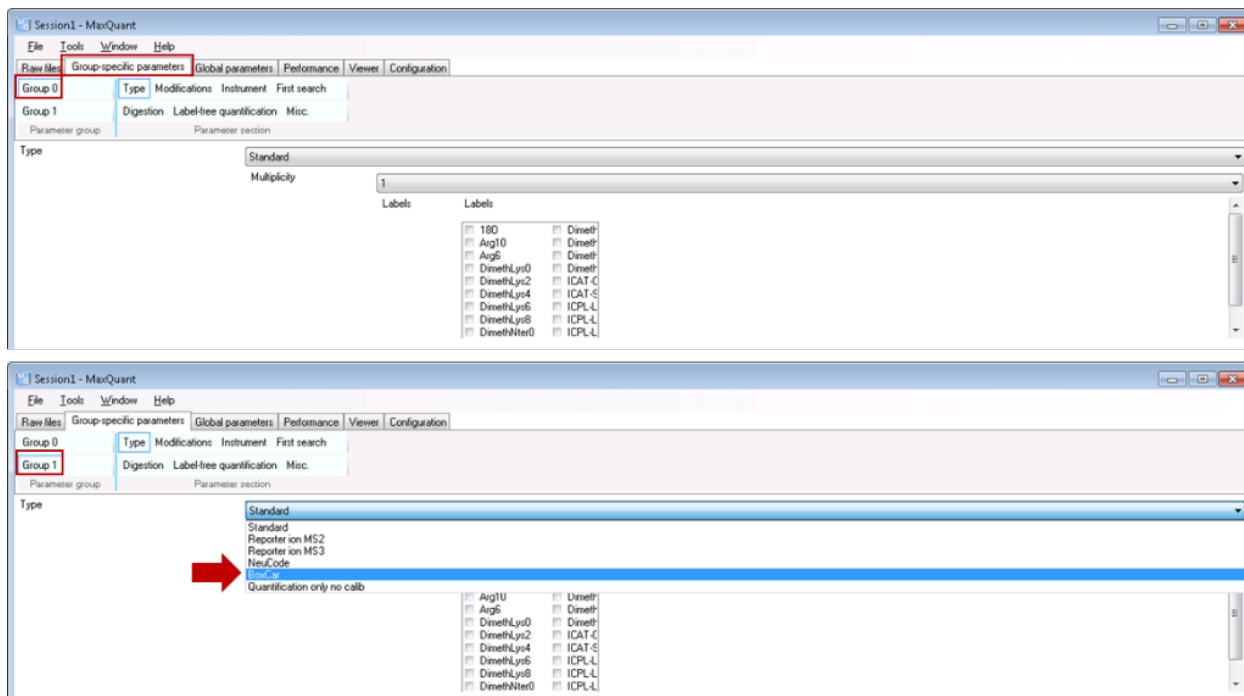
5. Set global parameters:

- Add the corresponding FASTA file(s) for the species under investigation.

- To transfer MS/MS identifications between runs, check "Match between runs" in the "Adv. identification" tab. It is recommended to adjust matching time tolerances according to your chromatography setup.

6. Start the automated processing workflow. MaxQuant output files will be written in the 'combinedtxt' folder when finished.

Easitag and TopN

The EasiTag strategy is based on the standard topN strategy but has some modifications and extensions. In EasiTag, the precursor selection is more stringent and only select monoisotopic peaks for fragmentation. Furthermore, the isolation windows are shifted by a global offset and their size chosen to be charge state dependent.

Method parameter	Description
General	
NumOfPrecursors	Number of precursor ions per acquisition cycle
DynamicExclusion	Switch for dynamic exclusion
ExclusionDuration	Dynamic exclusion time of precursors after first spectrum (s)
ExclusionThreshold	Mass tolerance for dynamic exclusion (ppm)
LifeTime	Max time a MS2 scan stays in the queue (ms)
Isolation window	Width of the precursor isolation window (Th)
MinCharge	Minimum charge state of the precursor
MaxCharge	Maximum charge state of the precursor
Intensity threshold	Precursor intensity threshold (cps)
MinMass	Minimum mass threshold for precursor ions (Da)
MzOffset	Offset that is added to the peak mz-value (Th)
UseChargeSpecificIsolationWindows	Switch to enable charge specific isolation windows
IsolationWindowList	Define isolation windows widths for every allowed charge state
ScanDataAsProfile	Profile (true) or centroid (false) spectra
MS1 Scan settings	
PositiveMode	Ion polarity
MaxIT	Maximum ion injection time (ms)
Resolution	MS resolving power at m/z 200
AGCtarget	AGC target value (charges)
MzRange	Scan range (m/z)
Ms2 scan settings	
MaxIT	Maximum ion injection time (ms)
Resolution	MS resolving power at m/z 200
NCE	Normalized collision energies (whitespace separated - only EasiTag)
AgcTarget	AGC target value (charges)
LowerMzBound	Lower Mz boundary for Ms2 spectra
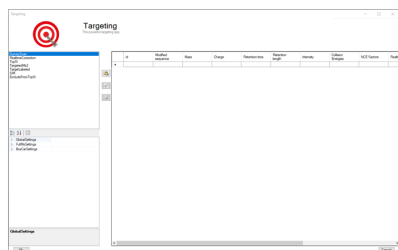
Targeting

MaxQuant.Live enables global targeting of more than 25,000 peptides
Christoph Wichmann, Florian Meier, Sebastian Virreira Winter, Andreas-David Brunner, Juergen Cox, Mat-thias Mann doi: 1101/443838

The Targeting app allows the realization of various targeting strategies within on user interface. First, the list of targeting peptides has to be populated and afterward the targeting strategy has to be defined and the parameters have to be set.

8.1 Steps to setup a targeting run

1. Populate the peptide list

2. Set up targeting action

3. Set global parameters

8.2 Peptide list

The targeting should comprise a set of peptides of interest and a set of high abundant peptides used for the real-time corrections of mass-to-charge values, expected retention times and peptide intensities. In order to get a reliable correction around 100 correction peptides per minute of the gradient is recommended. If the set of targeting peptides is large enough, it is sufficient to only use them as correction peptides.

Method parameter	Description	
Id	Identification number used in log messages	optional
Modified sequence	String containing the modified sequence	optional; Needed if isotopic labeling is used
Mass	Ion mass	mandatory
Charge	Ion charge state	mandatory
Retention time	Expected retention time (min)	mandatory
Retention length	Expected elution time (min)	mandatory but can be set to 0.0
Intensity	Expected intensity	mandatory but can be set to 1.0
Collision Energies	Collision energies for fragmentation (white space separated: 11 21)	optional; if empty the global NCE is used
NCE	List of NCE scaling factor for fragmentation (white space separated: 1.2 2.3)	optional;

The required information of every peptide is the mass, the expected retention time, the retention length and its charge state. Additionally the modified sequence should be provided to be able to find the peptide later in the MaxQuant.Live log file. The expected intensity completes the set of peptides properties. Note here, that the retention length is currently not taken into account and can therefore also be set to a fixed number like 1. If you don't have intensity information of the peptides it is also possible to set the intensities to one and choose the intensity threshold of the peptide recognition algorithm to a small value. The other columns contain checkboxes to enable actions called when the peptide is detected. The peptide table can either be populated manually or filled automatically by pasting a table from the clipboard. In the latter case, a tabular calculation program like Microsoft Excel can be used to set up the peptide list. Select the rows of peptides in Excel, copy to the clipboard and click on the paste icon in MaxQuant.Live to paste into the peptide list. Make sure that the header row is also copied and that it contains the same labels as the MaxQuant.Live peptide list. Only then MaxQuant.Live can map the columns correctly.

8.3 Setup of targeting actions

There are three ways to select targeting actions for peptides in the list 1. Click on the checkbox for every peptide manually 2. Select one or more peptides in the list, then select an action from the list of actions in the left panel and click on the check mark symbol in the middle. 3. Add a column with the name of the action to your peptide list in Excel and will it with the Boolean values `TRUE` or `FALSE`. When pasting the peptide in MaxQuant.Live, the software will set the checkboxes automatically.

8.3.1 RealtimeCorrection

The adaptive real-time correction adjusts the expected retention times based on the deviations of the peptide observed before. We refer to our article preprint in bioRxiv for more information about the real-time correction algorithm. We recommend to select around 100 peptides per minute ensure that the algorithm has enough statistic to take well-founded decisions. These peptide should be have a high intensity.

Parameter	Description	Default value
RetentionTimeWindow	Retention time window size [min]	20
MzWindowBig	Max mz window size [ppm]	20
MzWindowSmall	Min mz window size [ppm]	9
SigmaScaleFactorRt	Scaling factor for retention time window adjustment	3
PeptideHistoryLength	Time span considered to calculate the corrections [min]	2
MinHistoryPeptides	Minimum number of peptides used to calculate the corrections	50
IntensityThreshold		1.00E-05
PepdideDetectionIsoPeaks	Number of isotopic peaks that need to be found	2
IsotopeTolerance	Isotope mass tolerance [ppm]	9

8.3.2 TargetedMs2

Parameter	Description	Default value
BatMode	Fragment ion in every cycle within the retention time, even if not detected	FALSE
Prioritize	Prioritize MS2 scan, if the peptide was not fragmented before	TRUE
MaxNumOfScans	Upper limit for the number of fragmentations per peptide. 0=no limit	0
WindowSize	Size of the isolation window [th]	1.4
CollisionEnergy	Collision energy for fragmentation	27
LifeTime	Max time span before a scheduled MS2 scan get deleted [ms]	2000
MaxIT	Maximum ion injection time (ms)	28
Resolution	MS resolving power at m/z 200	15000
AgcTarget	AGC target value (charges)	100000
PositiveMode	Ion polarity	TRUE

8.3.3 TargetLabeled

This action adds the SILAC label masses to the peptides masses in the list. The "Modified is used to calculate the number Arg and Lys amino acids. The masses of SILAC labels can be freely defined here.

8.3.4 SIM (Selected Ion Monitoring)

Parameter	Description	Default value
WindowOffset	Shift the isolation window centers by this offset [th]	0
Isotopic label	Masses of SILAC labels	
MaxIT	Maximum ion injection time (ms) [not used]	50
MaxItLight /Heavy	Maximum ion injection time for light/heavy channel (ms)	100
InjectTarget-Light	AGC target value for light/heavy peptide (charges)	50000
BatMode	Fragment ion in every cycle within the retention time tolerance window, even if not detected	FALSE
Prioritize	Prioritize MS2 scan, if the peptide was not fragmented before	TRUE
MaxNumOfS-cans	Upper limit for the number of fragmentations per peptide. 0=no limit	0
WindowSize	Size of the isolation windows [th]	1.4
CollisionEnergy	Collision energy for fragmentation	0
LifeTime	Max time span before a scheduled MS2 scan get deleted [ms]	1000
Resolution	MS resolving power at m/z 200	30000
AgcTarget	AGC target value (charges)	100000
PositiveMode	Ion polarity	TRUE

8.3.5 ExcludeFromTopN

Parameter	Description	Default value
BatMode	Exclude ion from TopN within the retention time tolerance window , even if not detected	FALSE
StaticExclusion-Time	Time span the peptide is excluded from TopN (30)	30
AdaptiveExclusion-Time	Automatic exclusion of the peptide from TopN	TRUE

8.4 Global Parameters

8.4.1 SurveyScan

The survey scans for the peptide recognition can be either MS1 scan or BoxCar scan. The parameters are described in the BoxCar section.

8.4.2 TopN

The TopN strategy is applied on top of the targeting scans. Note, that peptides can also be excluded from being selected a precursor by using the "ExcludeFromTopN" feature.