

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS1-1	MS1 Signal	Ion injection times for IDs	MS1 median	ms	↓	Lower times indicate an abundance of ions	MS1 ion injection time

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

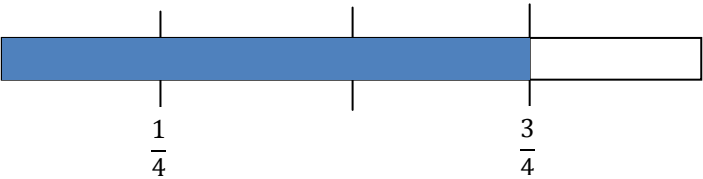
read in all the MS1 injection times

⇒ median injection time

MS1-2A	MS1 Signal	MS1 during middle (and early) peptide retention period	S/N median	None	↑	Higher MS1 S/N may correlate with higher signal discrimination	Median signal-to-noise value (ratio of maximum to median peak height) for MS1 spectra up to and including C-2A
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Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$



begin = first identified peptide

end = $\frac{3}{4}$ [total number of scans with identified peptides]

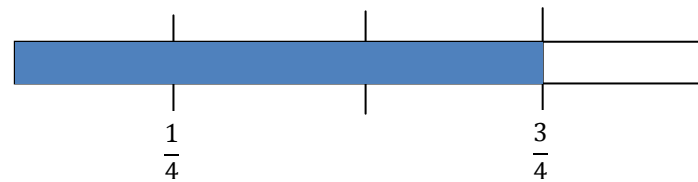
sort by MS1 intensities
read in BasePeakSignalToNoiseRatio values
BasePeakSignalToNoise found in ScanStats

⇒ median S/N

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS1-2B	MS1 Signal	MS1 during middle (and early) peptide retention period	TIC median	Counts/1000	↑	Estimates the total absolute signal for peptides (may vary significantly between instruments)	Median TIC value for identified peptides over same time period as used for MS1-2A

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$



begin = first identified peptide

end = $\frac{3}{4}$ [total number of scans with identified peptides]

store TotalIonIntensity values for MS1 scans
done over the retention spread
TotalIonIntensity found in ScanStats

\Rightarrow median TotalIonIntensity

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS1-3A	MS1 Signal	MS1 ID max	95/5 pctl	Ratio	↑	Estimates the dynamic range of the peptide signals	Ratio of 95 th over 5 th percentile MS1 maximum intensity values for identified peptides (approximates dynamic range of signal)

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

do for each peptide:

read in all the MS1 intensity values
use PeakMaxIntensities found in SICstats

sort the intensities
95th = 0.95(size)
5th = 0.05(size)

$$\Rightarrow \frac{95\text{th}}{5\text{th}}$$

MS1-3B	MS1 Signal	MS1 ID max	Median	Counts	↑	Estimates the median MS1 signal for peptides	Median maximum MS1 value for identified peptides
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Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

find the maximum MS1 intensity for each identified peptide
use PeakMaxIntensities found in SICstats

\Rightarrow median value of maximum MS1 values

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS1-4A	MS1 Signal	MS1 intensity variation for peptides	Within series	Percent	↓	Used to monitor relative intensity difference with a series	Average of between series intensity variations for identified peptides

Mathematical Definition:

* requires data from previous run *

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

Run	Peptide_Sequence	PeakMaxIntensity
1	A	50
1	A	75
1	A	70
1	B	100
2	A	72
2	B	110
2	B	105

if the peptide has multiple observations take the max PeakMaxIntensity value
use PeakMaxIntensities found in SICstats

Run	Peptide_Sequence	PeakMaxIntensity
1	A	50
1	A	75
1	A	70
1	B	100
2	A	72
2	B	110
2	B	105

⇒

Run	Peptide_Sequence	PeakMaxIntensity
1	A	75
1	B	100
2	A	72
2	B	110

to get the variation between the two runs, take the difference

Peptide_Sequence	Difference	Absolute Difference
A	$72 - 75 = -3$	3
B	$110 - 100 = 10$	10

average variation = $(3+10)/2 = 6.5$

⇒ average variation

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS1-4B	MS1 Signal	MS1 intensity variation for peptides	Betw/in	Ratio	↓	Used to monitor relative intensity differences with a series compared with between series	Ratio of average intensity variation between series to average intensity variation within a series (low values indicate similarity between series)
MS1-5A	MS1 Signal	Precursor m/z – Peptide ion m/z	Median	Th	↓	Measures the accuracy of the identifications	Median real value of precursor errors

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

$$mz - \frac{\text{Peptide_MH} - 1.00727649}{\text{charge}}$$

use mz found in SICstats

create an array of precursor m/z values with only unique peptides
 \Rightarrow return the median

MS1-5B	MS1 Signal	Precursor m/z – Peptide ion m/z	Mean absolute	Th	↓	Measures the accuracy of the identifications	Mean of the absolute precursor errors
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Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

$$\left| mz - \frac{\text{Peptide_MH} - 1.00727649}{\text{charge}} \right|$$

use mz found in SICstats

create an array of precursor m/z values with only unique peptides
 \Rightarrow return the absolute mean value

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS1-5C	MS1 Signal	Precursor m/z – Peptide ion m/z	ppm median	ppm	↓	Measures the accuracy of the identifications	Median real value of precursor errors in ppm

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

ppm \equiv parts per million

$$\text{difference} = m/z - \frac{\text{Peptide_MH} - 1.00727649}{\text{charge}}$$

calculate:

$$\frac{m/z}{\text{difference}} * 10^6$$

use m/z found in SICstats

create and sort an array with calculated values
 \Rightarrow return the median value

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS1-5D	MS1 Signal	Precursor m/z – Peptide ion m/z	ppm interQ	ppm	↓	Measures the distribution of the real accuracy measurements	Interquartile distance in ppm of the precursor errors

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

ppm \equiv parts per million

$$\text{difference} = m\text{z} - \frac{\text{Peptide_MH} - 1.00727649}{\text{charge}}$$

calculate:

$$\frac{m\text{z}}{\text{difference}} * 10^6$$

use mz found in SICstats

create and sort an array with calculated values

75th = 0.75(size)

25th = 0.25(size)

\Rightarrow 75th – 25th

MS2-1	MS2 Signal	Ion injection times for IDs	MS2 median	ms	↓		MS2 ion injection time
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Mathematical Definition:

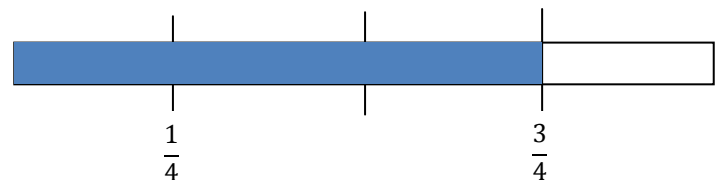
read in all the MS2 injection times
use scan type 2 or higher
ScanType found in ScanStats

\Rightarrow return median injection time

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS2-2	MS2 Signal	MS2 ID S/N	Median	Ratio	↑	Higher S/N correlates with increased frequency of peptide identification	Median S/N (ratio of maximum to median peak height) for identified MS2 spectra

Mathematical Definition:

filter first: Peptide_Expected_Value_Log(e) ≤ Max_Log_EValue = -2



begin = first identified peptide

end = $\frac{3}{4}$ [total number of scans with identified peptides]

sort by MS2 (or higher) intensities
read in BasePeakSignalToNoiseRatio values
BasePeakSignalToNoiseRaio found in ScanStats

⇒ median S/N

MS2-3	MS2 Signal	MS2 ID peaks	Median	Count	↑	Higher peak counts can correlate with more signal	Median number of peaks in an MS2 scan
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Mathematical Definition:

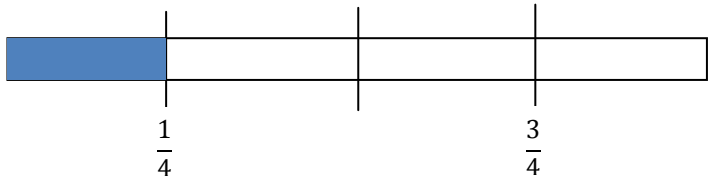
read in and sort IonCountRaw values for MS2 scans (or higher)
IonCountRaw found in ScanStats

⇒ median IonCountRaw value

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS2-4A	MS2 Signal	Fraction of MS2 identified at different MS1 max quartiles	ID fract Q1	Fraction	↑	Higher fractions of identified MS2 spectra indicate efficiency of detection and sampling	Fraction of total MS2 scans identified in the first quartile of peptides sorted by MS1 maximum intensity

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$



$$\text{begin} = \frac{1}{4} [\text{total number of MS2 scans}]$$

using scan type 2 or higher
use ScanType in ScanStats

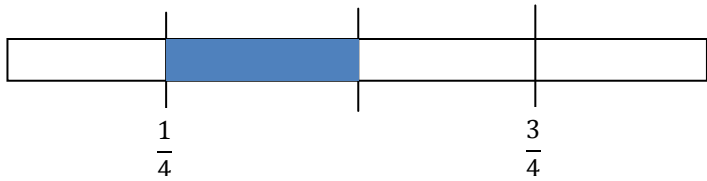
sort by MS1 maximum intensity

$$\Rightarrow \frac{\text{\# of scans identified in the begin segment}}{\text{total \# of scans}}$$

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS2-4B	MS2 Signal	Fraction of MS2 identified at different MS1 max quartiles	ID fract Q2	Fraction	↑	Higher fractions of identified MS2 spectra indicate efficiency of detection and sampling	Fraction of totalMS2 scans identified in the second quartile of peptides sorted by MS1 maximum intensity

Mathematical Definition:

filter first: Peptide_Expected_Value_Log(e) ≤ Max_Log_EValue = -2



$$\text{begin} = \frac{1}{4} [\text{total number of MS2 scans}]$$

$$\text{second} = \frac{1}{2} [\text{total number of MS2 scans}] - \text{begin}$$

using scan type 2 or higher
use ScanType in ScanStats

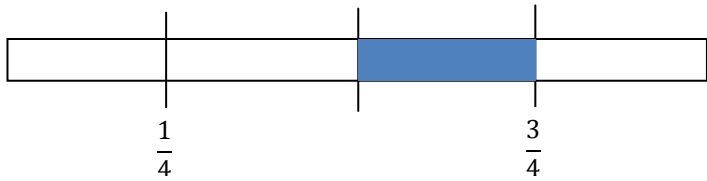
sort by MS1 maximum intensity

$$\Rightarrow \frac{\text{\# of scans identified in the second segment}}{\text{total \# of scans}}$$

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS2-4C	MS2 Signal	Fraction of MS2 identified at different MS1 max quartiles	ID fract Q3	Fraction	↑	Higher fractions of identified MS2 spectra indicate efficiency of detection and sampling	Fraction of totalMS2 scans identified in the third quartile of peptides sorted by MS1 maximum intensity

Mathematical Definition:

filter first: Peptide_Expected_Value_Log(e) ≤ Max_Log_EValue = -2



$$\text{third} = \frac{3}{4}[\text{total number MS2 scans}] - \frac{1}{2}[\text{total number of MS2 scans}]$$

using scan type 2 or higher
use ScanType in ScanStats

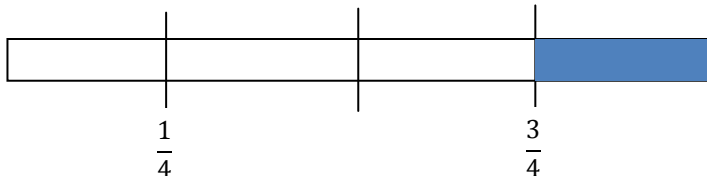
sort by MS1 maximum intensity

$$\Rightarrow \frac{\text{\# of scans identified in the third segment}}{\text{total \# of scans}}$$

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
MS2-4D	MS2 Signal	Fraction of MS2 identified at different MS1 max quartiles	ID fract Q4	Fraction	↑	Higher fractions of identified MS2 spectra indicate efficiency of detection and sampling	Fraction of totalMS2 scans identified in the last quartile of peptides sorted by MS1 maximum intensity

Mathematical Definition:

filter first: Peptide_Expected_Value_Log(e) ≤ Max_Log_EValue = -2



$$\text{end} = \text{total number of MS2 scans} - \frac{3}{4}[\text{total number of MS2 scans}]$$

using scan type 2 or higher
use ScanType in ScanStats

sort by MS1 maximum intensity

$$\Rightarrow \frac{\text{\# of scans identified in the end segment}}{\text{total \# of scans}}$$

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
P-1	Peptide Identification	MS2 ID score	Median	fval	↑	Higher scores correlate with higher S/N and frequency of identification	Median peptide identification score for all peptides; higher scores generally correlate with increased MS2 S/N

Mathematical Definition:

a.

read in and sort Peptide_Hyperscore values for entire run
Peptide_Hyperscore is found in xt

⇒ median Peptide_Hyperscore
higher value is better

b.

read in and sort Peptide_Expectation_Value_Log(e) values for entire run
Peptide_Expectation_Value_Log(e) is found in xt

⇒ median Peptide_Expectation_Value_Log(e)
lower value is better

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
P-2A	Peptide Identification	Tryptic peptide count	Identifications	Count	↑	Total identifications correlate with high levels of peptide signals, performance	Number of MS2 spectra identifying tryptic peptides ions (total “spectral counts”)

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

Scan	Charge	Peptide_Sequence	Cleavage_State
1	2	A	2
2	2	B	1
3	2	C	2
4	2	C	2
5	3	C	2
6	2	D	2
7	3	D	3
8	4	D	2
⋮	⋮	⋮	⋮
n-1	2	E	0
n	1	F	1

take the max of the Cleavage_State for same peptides sequence
count the number of peptides with Cleavage_State 2

count peptides with the same sequence only once
Cleavage_State is found in xt_SeqToProteinMap

\Rightarrow count

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
P-2B	Peptide Identification	Tryptic peptide count	Ions	Counts	↑	A good overall performance measure	Number of tryptic peptide ions identified; ions differing by charge state and/or modification state are counted separately

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

Scan	Charge	Peptide_Sequence	Cleavage_State
1	2	A	2
2	2	B	1
3	2	C	2
4	2	C	2
5	3	C	2
6	2	D	2
7	3	D	3
8	4	D	2
⋮	⋮	⋮	⋮
n-1	2	E	0
n	1	F	1

take the max of the Cleavage_State for same peptides sequence
count the number peptides with Cleavage_State 2 and different charges
if the peptide has Cleavage_State 2 and charge 2 and charge 3 count it twice

count peptides with the same sequence only once for each charge
Cleavage_State is found in xt_SeqToProteinMap
Charge is found in xt

⇒ count

link Charge to Result_ID in xt
link Result_ID found in xt to Result_ID xt_ResultToSeqMap
link Result_ID in xt_ResultToSeqMap to Unique_Seq_ID in xt_SeqToProteinMap
link Unique_Seq_ID in xt_ResultToSeqMap to Cleavage_State in xt_SeqToProteinMap

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
P-2C	Peptide Identification	Tryptic peptide counts	Peptides	Count	↑	A good overall performance measure	Number of unique tryptic peptide sequences identified

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

Scan	Charge	Peptide_Sequence	Cleavage_State
1	2	A	2
2	2	B	1
3	2	C	2
4	2	C	2
5	3	C	2
6	2	D	2
7	3	D	3
8	4	D	2
⋮	⋮	⋮	⋮
n-1	2	E	0
n	1	F	1

take the max of the Cleavage_State for same peptides sequence
count the number of unique peptides

count peptides with the same sequence only once
Cleavage_State is found in xt_SeqToProteinMap
use Peptide_Sequence is found in xt

\Rightarrow count

Cleavage_State is found in xt_SeqToProteinMap
Cleavage_State is found in xt_SeqToProteinMap

Code	Category	Metric Group	Metric	Units	Optimal	Purpose/Use	Description
P-3	Peptide Identification	Peptide counts	Semi/tryptic peptides	Ratio		Indicates prevalence of semitryptic peptides in sample; increasing ratios may indicate changes in sample or in source	Ratio of semi/full tryptic peptides

Mathematical Definition:

filter first: $\text{Peptide_Expected_Value_Log}(e) \leq \text{Max_Log_EValue} = -2$

take the max of the Cleavage_State for same peptides sequence
count peptides with the same sequence only once
Cleavage_State is found in xt_SeqToProteinMap

$$\Rightarrow \frac{\text{total \# of semi tryptic peptides}}{\text{total \# of tryptic peptides}}$$