Supporting Information for: Evaluating the Characteristics of Reporter Ion Signal Acquired in the Orbitrap Analyzer for Isobaric Mass Tag Proteome Quantification Experiments

Christopher S Hughes[1], Chenchen Zhu[2], Victor Spicer[3], Oleg V Krokhin[3,4], Gregg B Morin[1,5,*]

1 – Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, British Columbia, Canada

2 – European Molecular Biology Laboratory, Meyerhofstrasse 1, Heidelberg, Germany

3 – Manitoba Centre for Proteomics and Systems Biology, University of Manitoba, Winnipeg, Manitoba, Canada

4 – Department of Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

5 – Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

* To whom correspondence should be addressed: gmorin@bcgsc.ca

Supporting Information Table of Contents

Supporting Information Figure Legends

Figure S-1 – There was no observable relationship between the notch and the properties of precursor ions selected during MS acquisition. Representative raw data files obtained from in-depth quantitative analysis of 6 ovarian carcinoma cell lines were re-analyzed [1]. The data contains a total of 221,648 MS^3 scans where a value is reported in the TMT126 channel. From these, 15,000 scans were randomly selected and the data plotted for **(a)** scan number, **(b)** total ion current, **(c)** precursor mass-to-charge, **(d)** ion injection time against the reporter ion counts. Red arrowheads denote the notch region observed in the data.

Figure S-2 – The notch was observed in published data from independent Orbitrap Fusion MS instruments. Representative raw data files from ProteomeXchange of published TMT proteome quantification studies using an Orbitrap Fusion with MS^3 scanning were analyzed. The figure displays histograms of the TMT126 reporter ion counts obtained [2–11]. The text above each plot denotes the leading author on the original study (Table S-4). Values in inset red boxes display the proportion of values observed above the notch region. Dashed blue lines indicate the mean reporter ion counts for each ion channel.

Figure S-3 – The notch was observed in published data from other hybrid linear ion trap-Orbitrap MS instrument types. Representative raw data files from from ProteomeXchange of published studies using non-Fusion Orbitrap instruments with MS^3 scanning were analyzed [6,8,11]. The figure displays

histograms of the TMT126 reporter ion counts. The text above each plot denotes the leading author on the original study and the MS instrument type on which the data was acquired. Values in inset red boxes display the proportion of values observed above the notch region. Dashed blue lines indicate the mean reporter ion counts for each ion channel.

Figure S-4 – The notch was observed in published data from quadrupole Orbitrap MS instrument types. Data generated on a qExactive HF where TMT quantification was used were downloaded from ProteomeXChange (Accession: PXD004540) [12]. TMT data were parsed from the files using scripts in R based on the mzR package. Histogram plots display reporter ion **(a)** intensities and **(b)** ion counts. Inset boxes display the proportion of scans that are contained above the notch region in both data sets.

Figure S-5 – The notch was not observable in MS^3 data obtained using the IT analyser from an independent Orbitrap Fusion MS. Representative raw data files were queried from a recent published study [10]. Scans from a total of 5 technical replicate injections were compiled into a single plot. Shown are histograms of TMT128 reporter ion **(a)** ion counts and **(b)** intensities with an expected ratio of 10:2:1:1:2:10. The TMT128 channel is plotted as it represents the expected lowest intensity, and thus is the highest probability to enable visualization of the notch. Inset text indicates the IT-MS^3 scan settings utilized in the original study. Dashed blue lines indicate the mean reporter ion intensity for each ion channel.

Figure S-6 – The proportion of scans above the notch region decreased as the quantity of material analysed was reduced. A pool derived from a tryptic digest of 13 individual cell lines was TMT labeled (1:1:1:1:1:1 TMT126 – TMT131) and spiked with a mixture of 550 synthetic peptides (3:3:3:1:1:1 TMT126 – TMT131) and analyzed using 1.0 µg, 0.5µg, and 0.05µg on-column injection loads. The line plot depicts the proportion of scans that fall above the region of the notch across different on-column loaded quantities of peptide material. Values were calculated as a proportion of all scans in an analysis above the minimum value of total scans in a histogram bin found in the notch region.

Figure S-7 – The proportion of scans below the notch region could be reduced through modulation of the OT-MS^3 scan parameters. A pool derived from a tryptic digest of 13 individual cell lines was TMT labeled (1:1:1:1:1:1 TMT126 – TMT131) and spiked with a mixture of 550 synthetic peptides (3:3:3:1:1:1 TMT126 – TMT131) and analyzed using a selection of OT analyzer ion fill and time settings. Histogram plots of the representative TMT126 quantification channel for an automatic gain control setting of 2e4 and 2e5. Separate histograms are provided for MS^3 scans where the maximum ion injection time was reached. Maximum injection times are listed along the vertical axis. All reporter ion signal values are represented as ion counts. Inset values in red boxes indicate the proportion of scans found above the notch region.

Figure S-8 – The proportion of scans above the notch was increased when the signal-to-noise of the OT analyzer was improved. A pool derived from a tryptic digest of 13 individual cell lines was TMT labeled (1:1:1:1:1:1 TMT126 –

TMT131) and spiked with a mixture of 550 synthetic peptides (3:3:3:1:1:1 TMT126 – TMT131) and analyzed using 1.0µg on-column injection loads. The line plot depicts the proportion of scans that fell above the region of the notch based on reporter ion counts across different OT scan settings for maximum ion injection and fill times. Values were calculated as a proportion of all scans in an analysis above the minimum value of total scans in a histogram bin found in the notch region.

Figure S-9 – Manipulation of other user-adjustable method parameters did not alter observation of the notch in reporter ion data. A pool derived from a tryptic digest of 13 individual cell lines was TMT labeled (1:1:1:1:1:1 TMT126 – TMT131) and spiked with a mixture of 550 synthetic peptides (3:3:3:1:1:1 TMT126 – TMT131) and analyzed using 1.0µg on-column injection loads while adjusting isolation and selection MS parameters. **(a)** Histogram of reporter ion counts when an intensity filter was used for MS^3 ion selection (method A14). **(b)** Histogram of reporter ion counts when a MS^2 isolation window of 1 Da was used (method A11). **(c)** Histogram of reporter ion counts when a MS^3 isolation window of 1 Da was used (method A12). **(d)** Histogram of reporter ion counts when selection of 5 precursors in SPS-MS^3 was used (method A13).

Supporting Information Table Legends

Table S-1 – Synthetic peptide library. Contains all sequences synthesized for the synthetic peptide library.

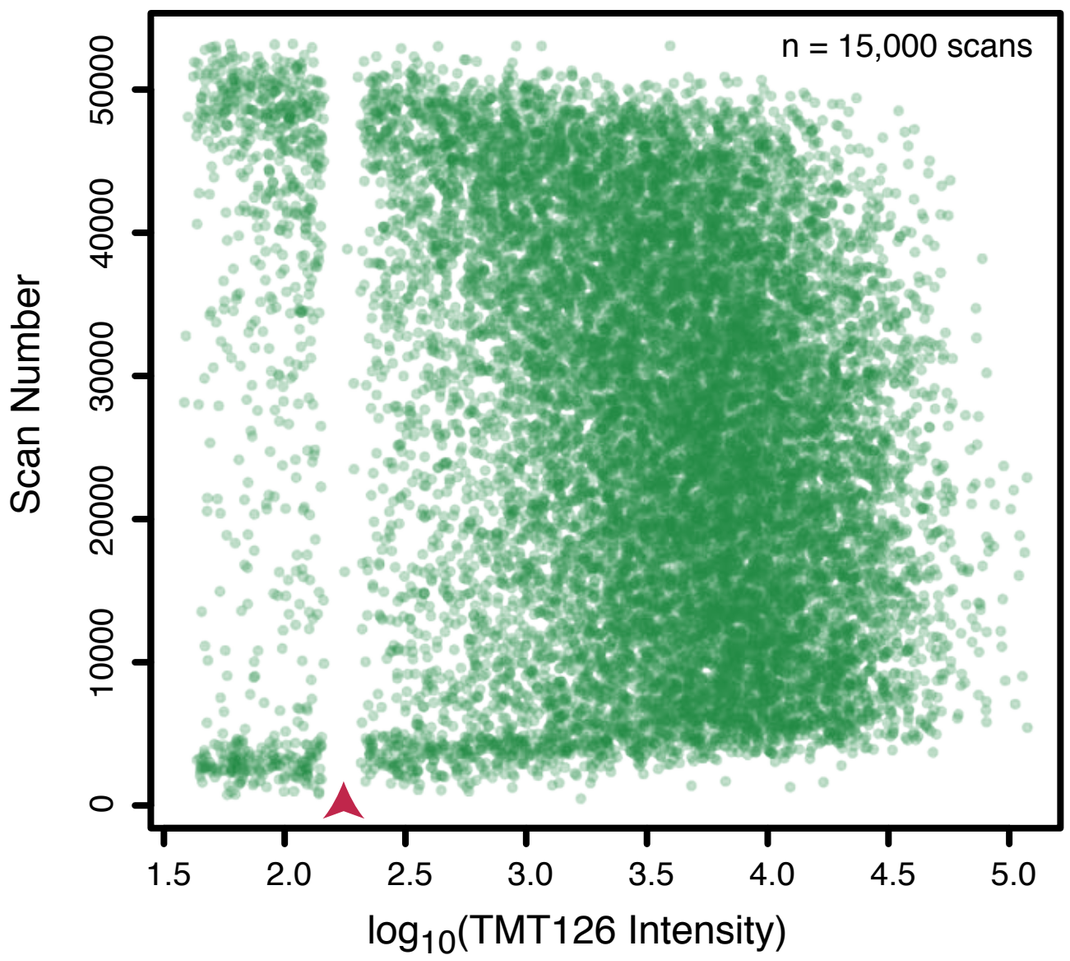
Table S-2 – Acquisition method settings for all experiments carried out in this work. Individual method settings for each experiment can be found by their referred code from the main text and tables. The method parameters are provided in the order they appear in the method editor layout. Each set of parameters is separated based on belonging to individual method tabs in the editor.

Table S-3 – Detailed information on R scripts provided with this work. Individual R scripts and their associated experiments are listed. All scripts are available as described in the main manuscript 'Data and Code Availability' section of the Methods.

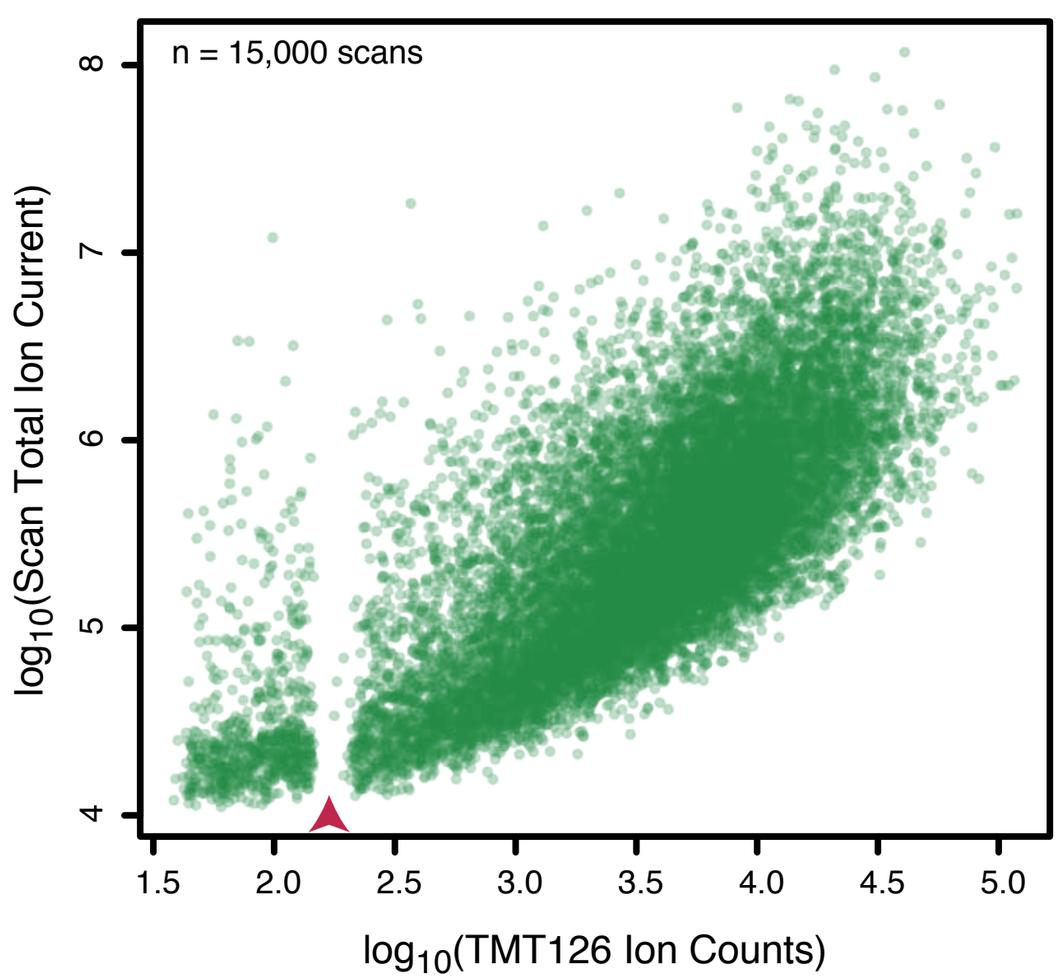
Table S-4 – Listing and descriptions of data sets used in this manuscript. All data files were selected randomly from the respective studies. A total of 3 individual raw files were analyzed for each study, unless noted otherwise.

Figure S-1

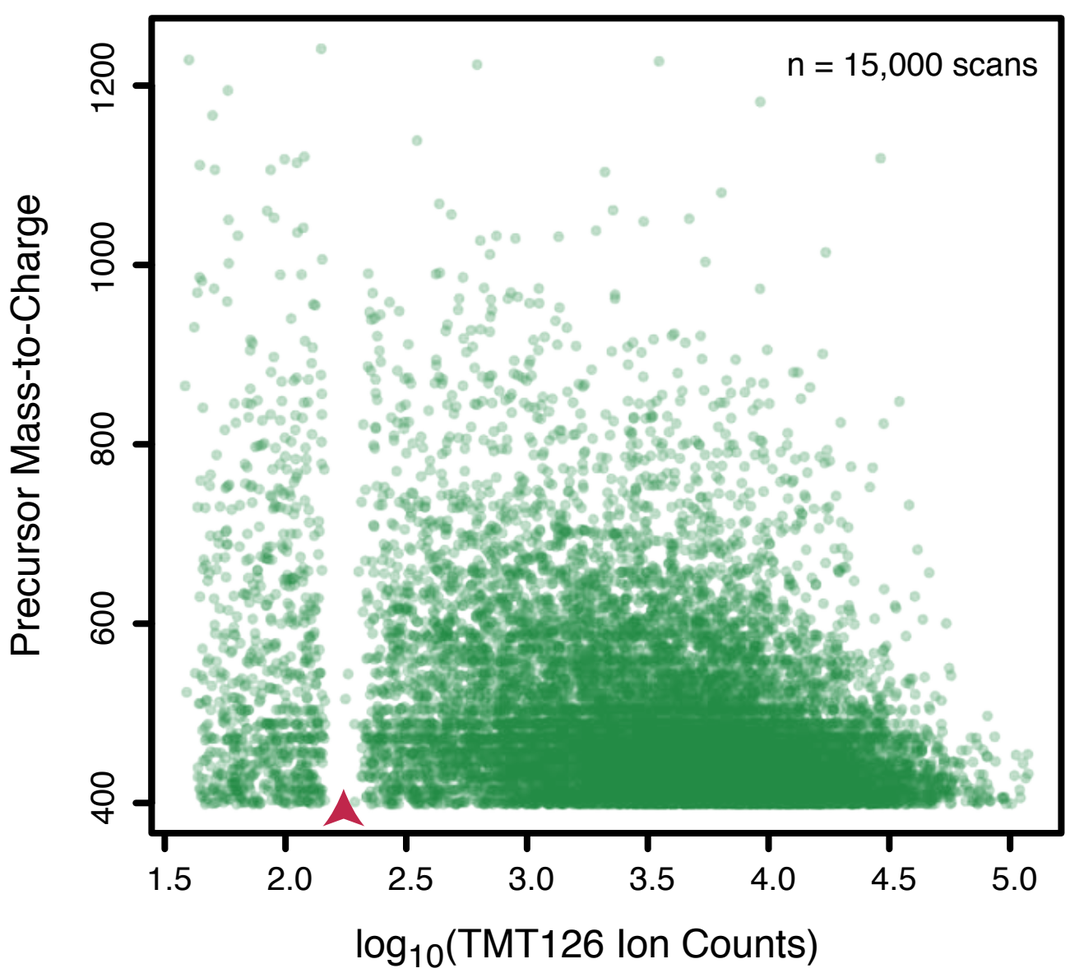
(a)



(b)



(c)



(d)

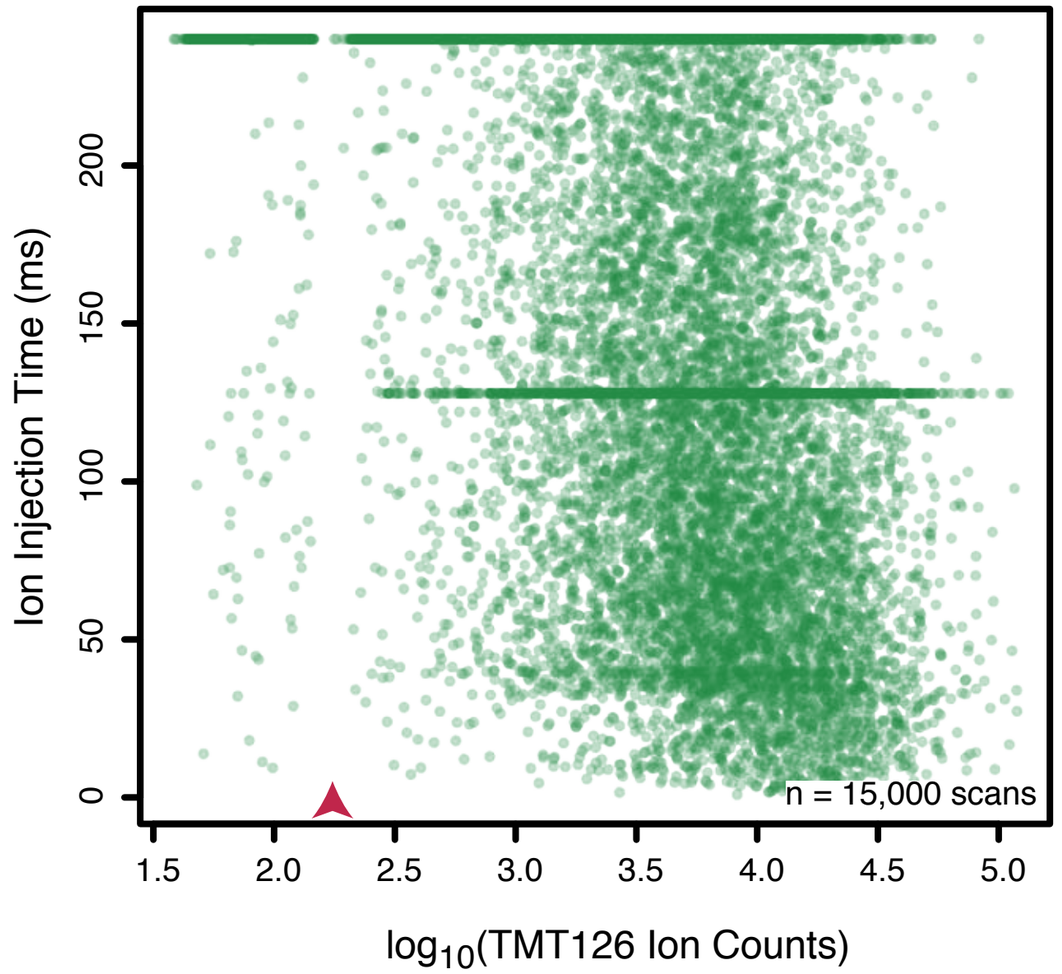


Figure S-2

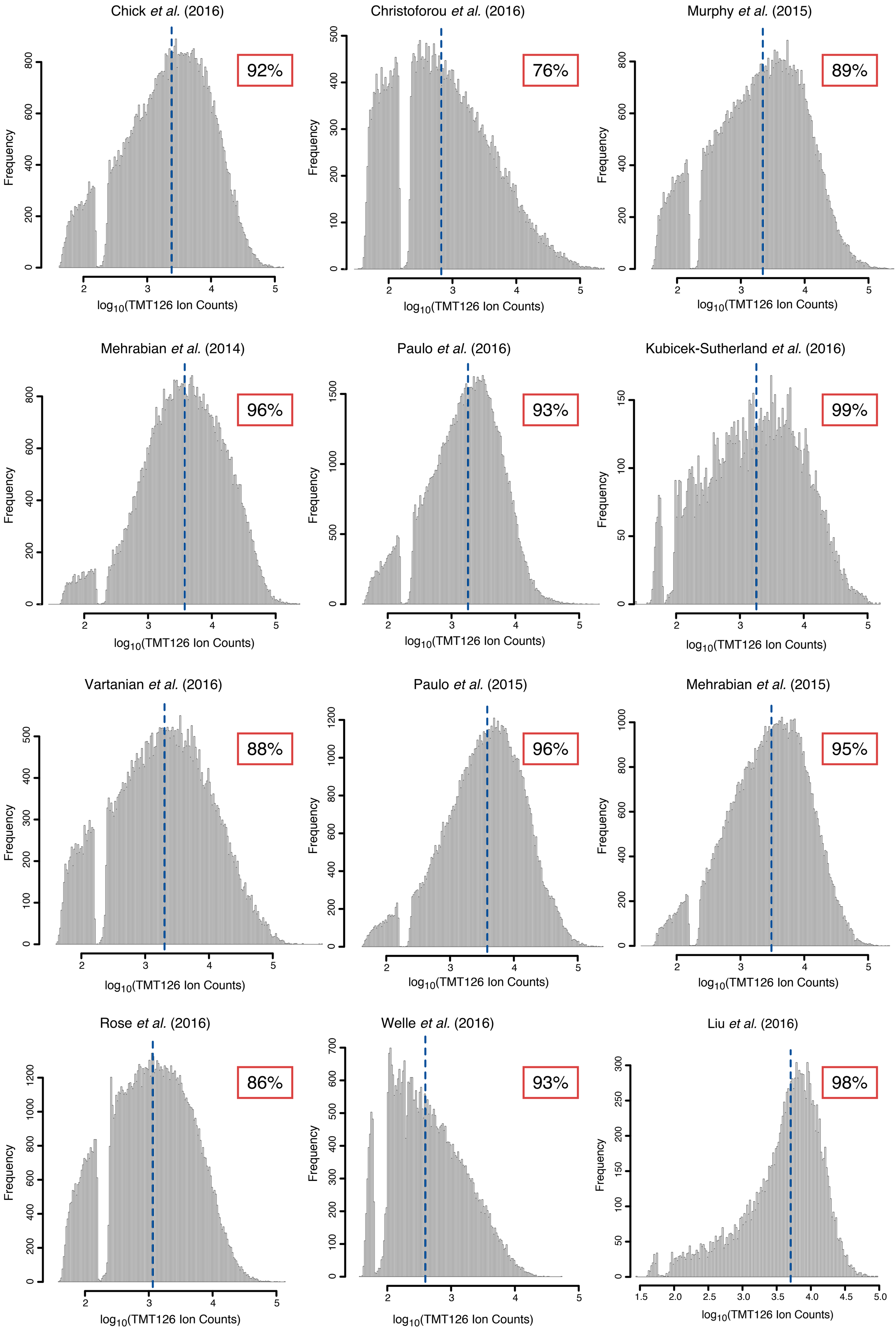

Chick et al. (2016)
92%
Christoforou et al. (2016)
76%
Murphy et al. (2015)
89%
Mehrabian et al. (2014)
96%
Paulo et al. (2016)
93%
Kubicek-Sutherland et al. (2016)
99%
Vartanian et al. (2016)
88%
Paulo et al. (2015)
96%
Mehrabian et al. (2015)
95%
Rose et al. (2016)
86%
Welle et al. (2016)
93%
Liu et al. (2016)
98%
Frequency
log10(TMT126 Ion Counts)

Figure S-3

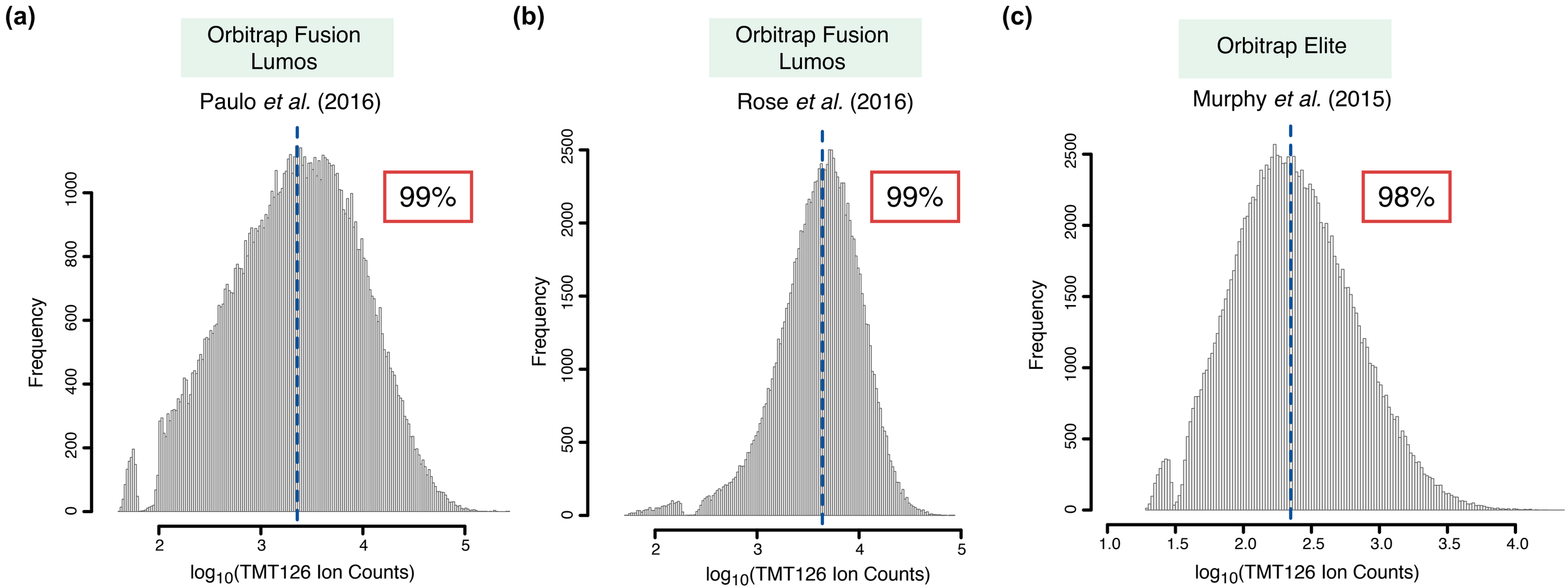

(a)
Orbitrap Fusion Lumos
Paulo et al. (2016)
99%
Frequency
0
200
400
600
800
1000
2
3
4
5
log10(TMT126 Ion Counts)
(b)
Orbitrap Fusion Lumos
Rose et al. (2016)
99%
Frequency
0
500
1000
1500
2000
2500
2
3
4
5
log10(TMT126 Ion Counts)
(c)
Orbitrap Elite
Murphy et al. (2015)
98%
Frequency
0
500
1000
1500
2000
2500
1.0
1.5
2.0
2.5
3.0
3.5
4.0
log10(TMT126 Ion Counts)

Figure S-4

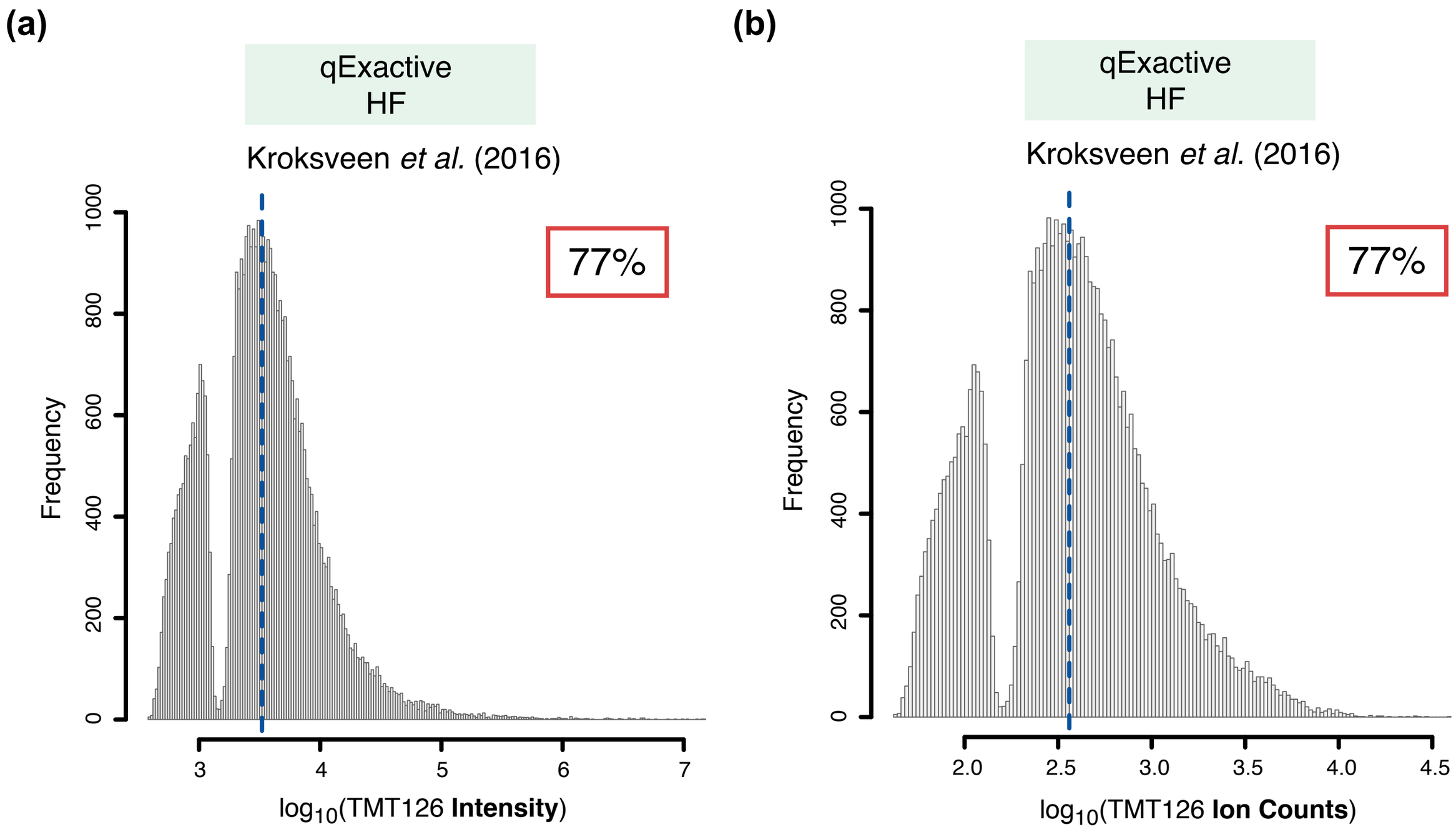

(a)
qExactive
HF
Kroksveen *et al.* (2016)
77%
Frequency
0
200
400
600
800
1000
3
4
5
6
7
log10(TMT126 **Intensity**)
(b)
qExactive
HF
Kroksveen *et al.* (2016)
77%
Frequency
0
200
400
600
800
1000
2.0
2.5
3.0
3.5
4.0
4.5
log10(TMT126 **Ion Counts**)

Figure S-5

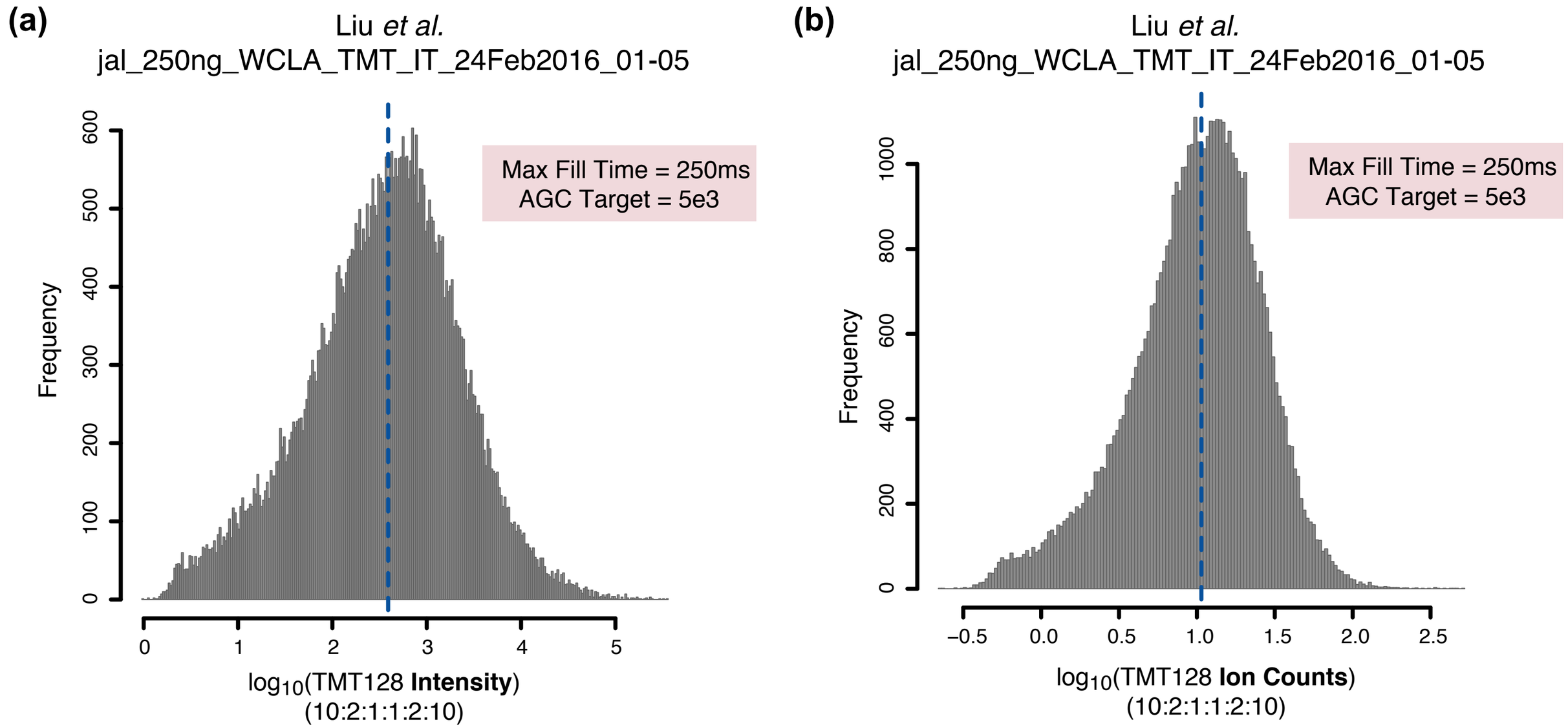

(a)
Liu et al.
jal_250ng_WCLA_TMT_IT_24Feb2016_01-05
Max Fill Time = 250ms
AGC Target = 5e3
Frequency
0
100
200
300
400
500
600
0
1
2
3
4
5
log10(TMT128 Intensity)
(10:2:1:1:2:10)
(b)
Liu et al.
jal_250ng_WCLA_TMT_IT_24Feb2016_01-05
Max Fill Time = 250ms
AGC Target = 5e3
Frequency
0
200
400
600
800
1000
−0.5
0.0
0.5
1.0
1.5
2.0
2.5
log10(TMT128 Ion Counts)
(10:2:1:1:2:10)

Figure S-6

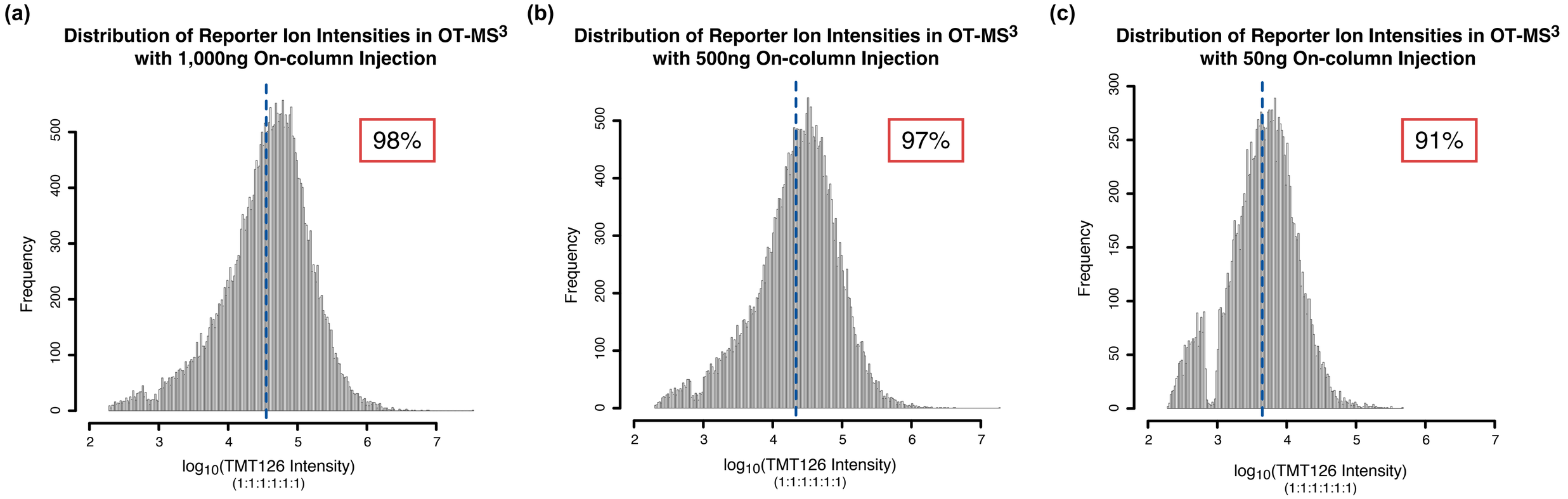

(a)
Distribution of Reporter Ion Intensities in OT-MS3
with 1,000ng On-column Injection
98%
Frequency
0
100
200
300
400
500
2
3
4
5
6
7
log10(TMT126 Intensity)
(1:1:1:1:1:1)
(b)
Distribution of Reporter Ion Intensities in OT-MS3
with 500ng On-column Injection
97%
Frequency
0
100
200
300
400
500
2
3
4
5
6
7
log10(TMT126 Intensity)
(1:1:1:1:1:1)
(c)
Distribution of Reporter Ion Intensities in OT-MS3
with 50ng On-column Injection
91%
Frequency
0
50
100
150
200
250
300
2
3
4
5
6
7
log10(TMT126 Intensity)
(1:1:1:1:1:1)

Figure S-7

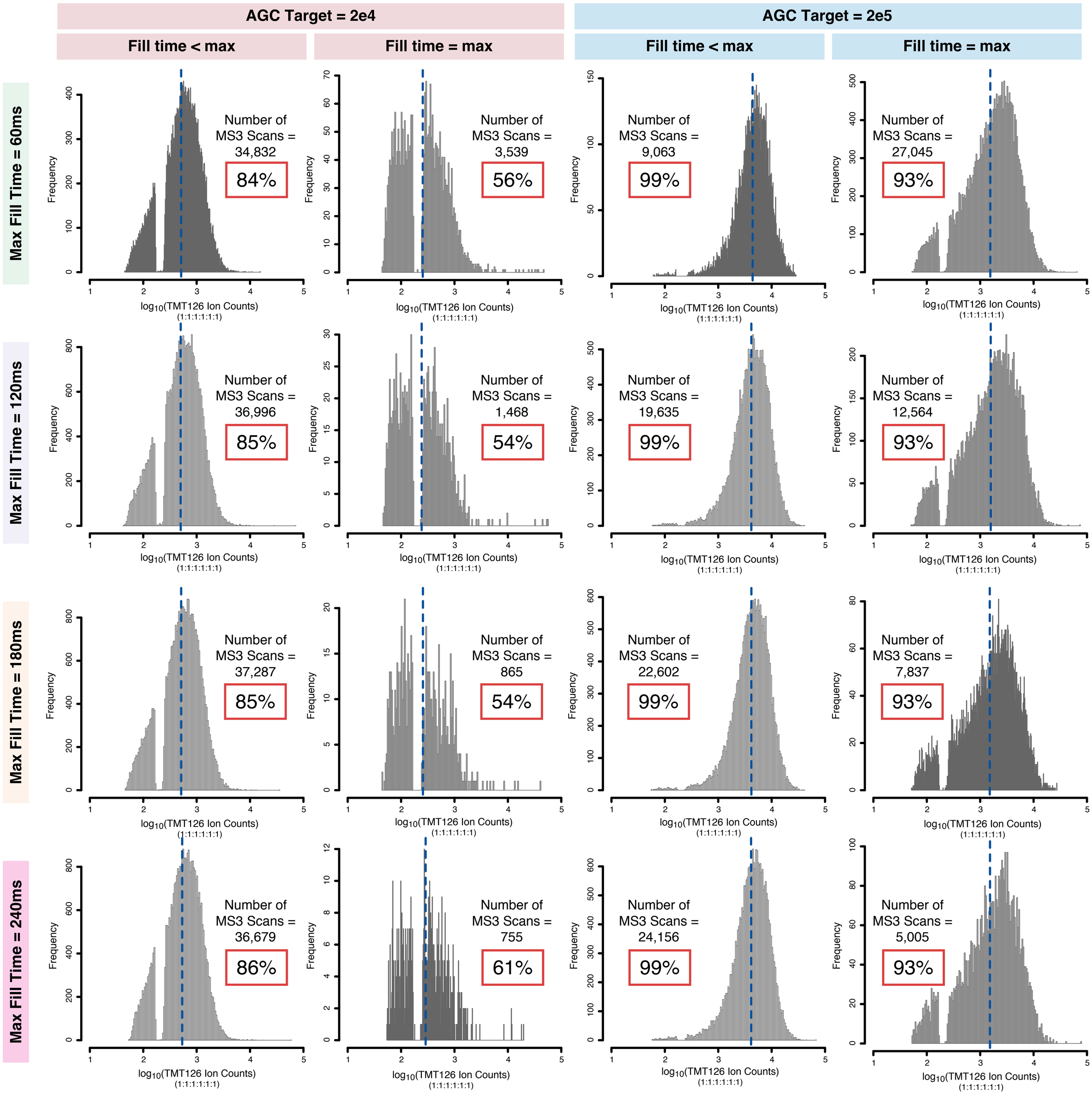

AGC Target = 2e4
AGC Target = 2e5
Fill time < max
Fill time = max
Fill time < max
Fill time = max
Max Fill Time = 60ms
Max Fill Time = 120ms
Max Fill Time = 180ms
Max Fill Time = 240ms
Number of MS3 Scans = 34,832
84%
Number of MS3 Scans = 3,539
56%
Number of MS3 Scans = 9,063
99%
Number of MS3 Scans = 27,045
93%
Number of MS3 Scans = 36,996
85%
Number of MS3 Scans = 1,468
54%
Number of MS3 Scans = 19,635
99%
Number of MS3 Scans = 12,564
93%
Number of MS3 Scans = 37,287
85%
Number of MS3 Scans = 865
54%
Number of MS3 Scans = 22,602
99%
Number of MS3 Scans = 7,837
93%
Number of MS3 Scans = 36,679
86%
Number of MS3 Scans = 755
61%
Number of MS3 Scans = 24,156
99%
Number of MS3 Scans = 5,005
93%
Frequency
log10(TMT126 Ion Counts)
(1:1:1:1:1:1:1:1)

Figure S-8

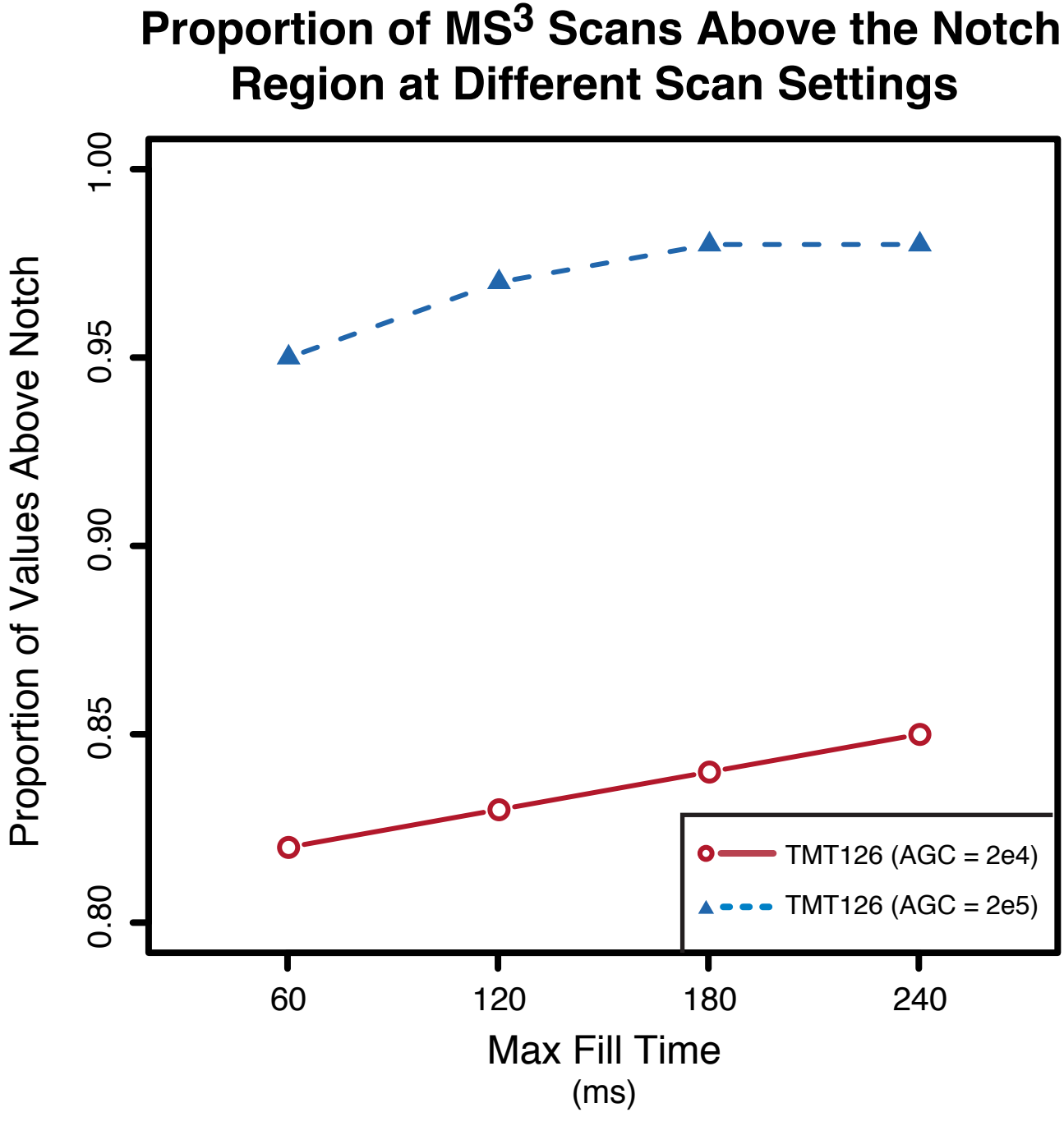
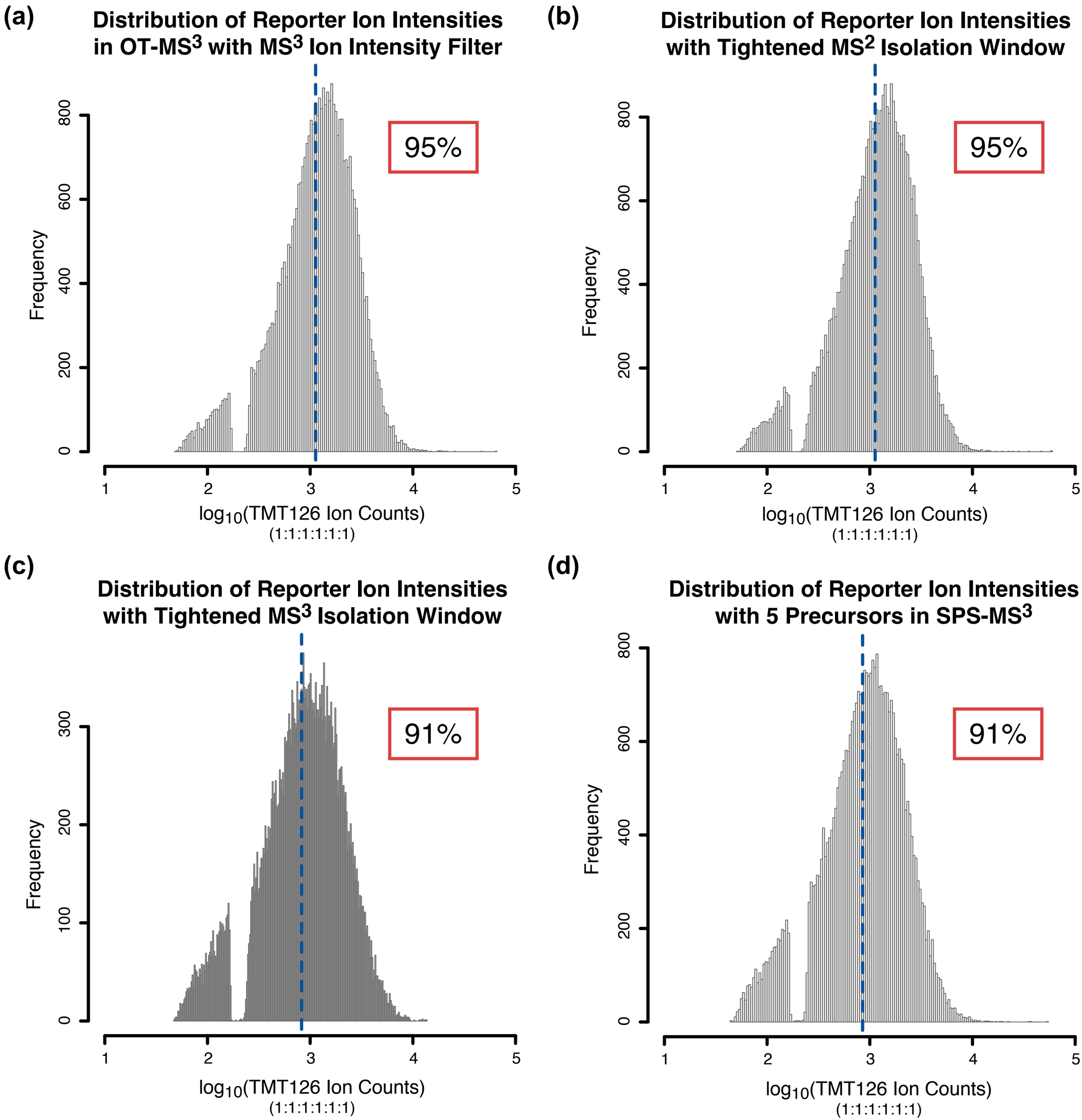

Proportion of MS3 Scans Above the Notch Region at Different Scan Settings
Proportion of Values Above Notch
1.00
0.95
0.90
0.85
0.80
60
120
180
240
Max Fill Time
(ms)
TMT126 (AGC = 2e4)
TMT126 (AGC = 2e5)

Figure S-9


(a)
Distribution of Reporter Ion Intensities in OT-MS3 with MS3 Ion Intensity Filter
95%
Frequency
0
200
400
600
800
1
2
3
4
5
log10(TMT126 Ion Counts)
(1:1:1:1:1:1)
(b)
Distribution of Reporter Ion Intensities with Tightened MS2 Isolation Window
95%
Frequency
0
200
400
600
800
1
2
3
4
5
log10(TMT126 Ion Counts)
(1:1:1:1:1:1)
(c)
Distribution of Reporter Ion Intensities with Tightened MS3 Isolation Window
91%
Frequency
0
100
200
300
1
2
3
4
5
log10(TMT126 Ion Counts)
(1:1:1:1:1:1)
(d)
Distribution of Reporter Ion Intensities with 5 Precursors in SPS-MS3
91%
Frequency
0
200
400
600
800
1
2
3
4
5
log10(TMT126 Ion Counts)
(1:1:1:1:1:1)