

SUPPLEMENTARY FIGURES

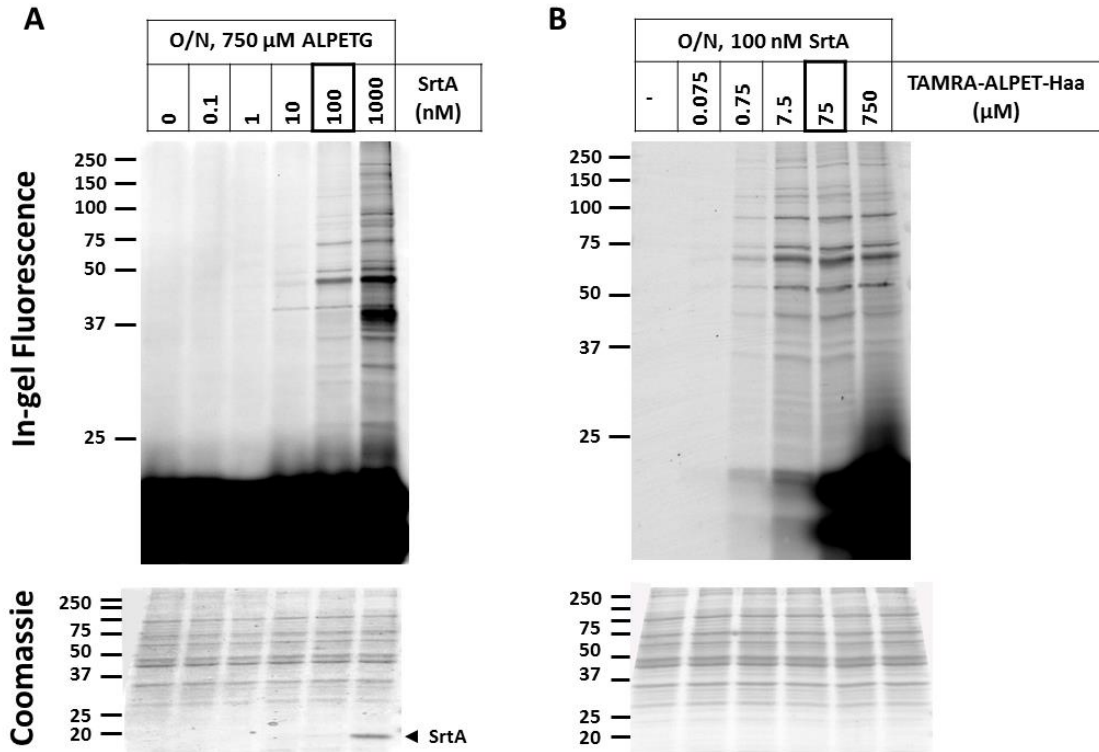


Figure S1. Optimization of overnight SrtA labeling reaction in whole-cell lysates. MDA-MB-231 cells were incubated with 100 nM IMP-1088 for 24 h before lysis. Lysates were labeled with SrtA and TAMRA-ALPET-Haa overnight at 4 °C and subjected to SDS-PAGE. In-gel fluorescence images were acquired with excitation/emission wavelengths of 532/568 nm. **(A)** Optimization of SrtA concentration with constant depsipeptide substrate. **(B)** Optimization of TAMRA-ALPET-Haa concentration with constant SrtA concentration. Highlighted are the concentrations selected for further experiments.

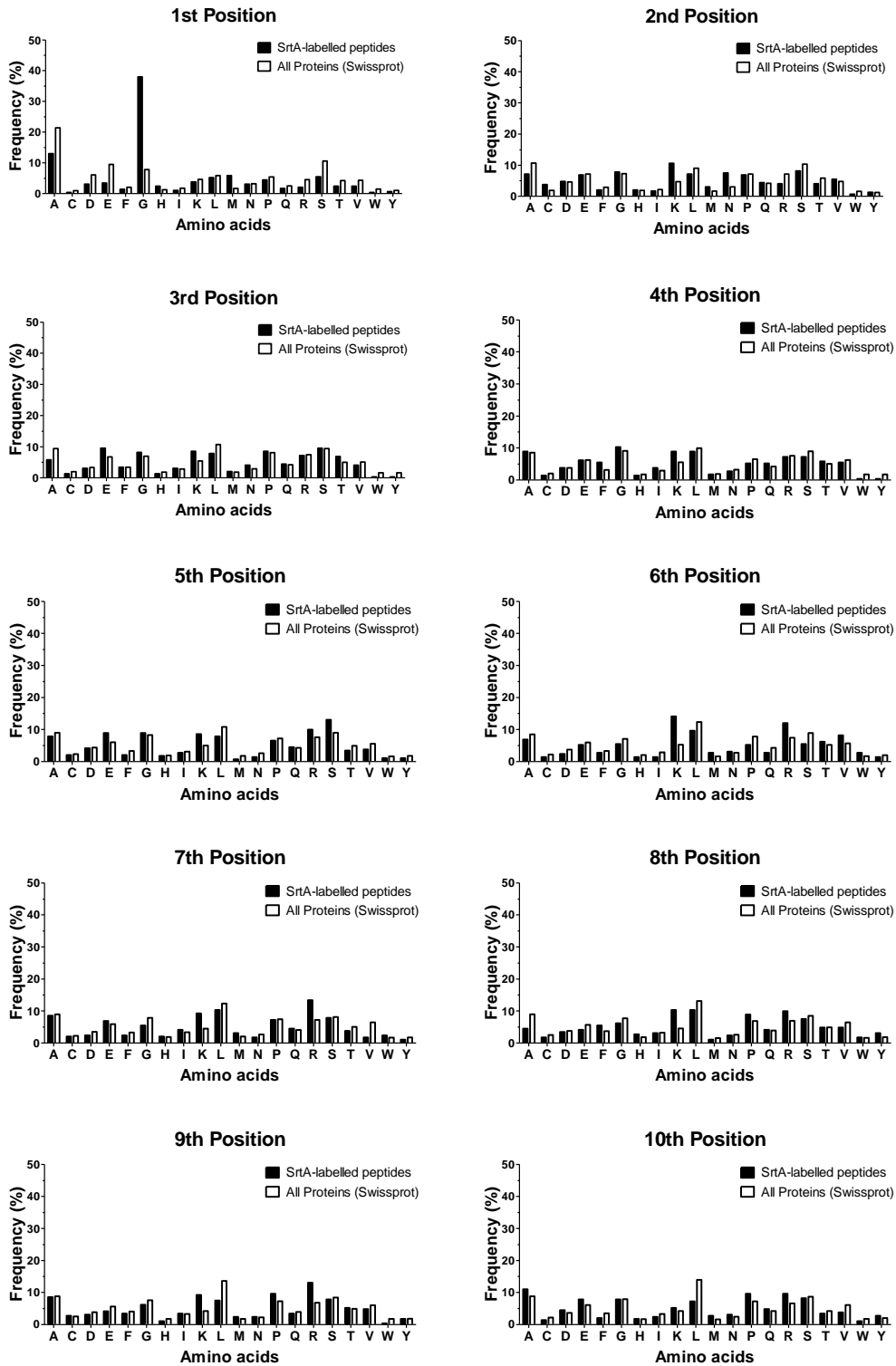


Figure S2. Bar plots comparing the frequencies for every amino acid per position in experimentally identified SrtA-labeled peptides and for all peptides of the human proteome (Swissprot, Uniprot, December 2017; 42,326 entries).

Raw File
agg_pep_sort_pos_1

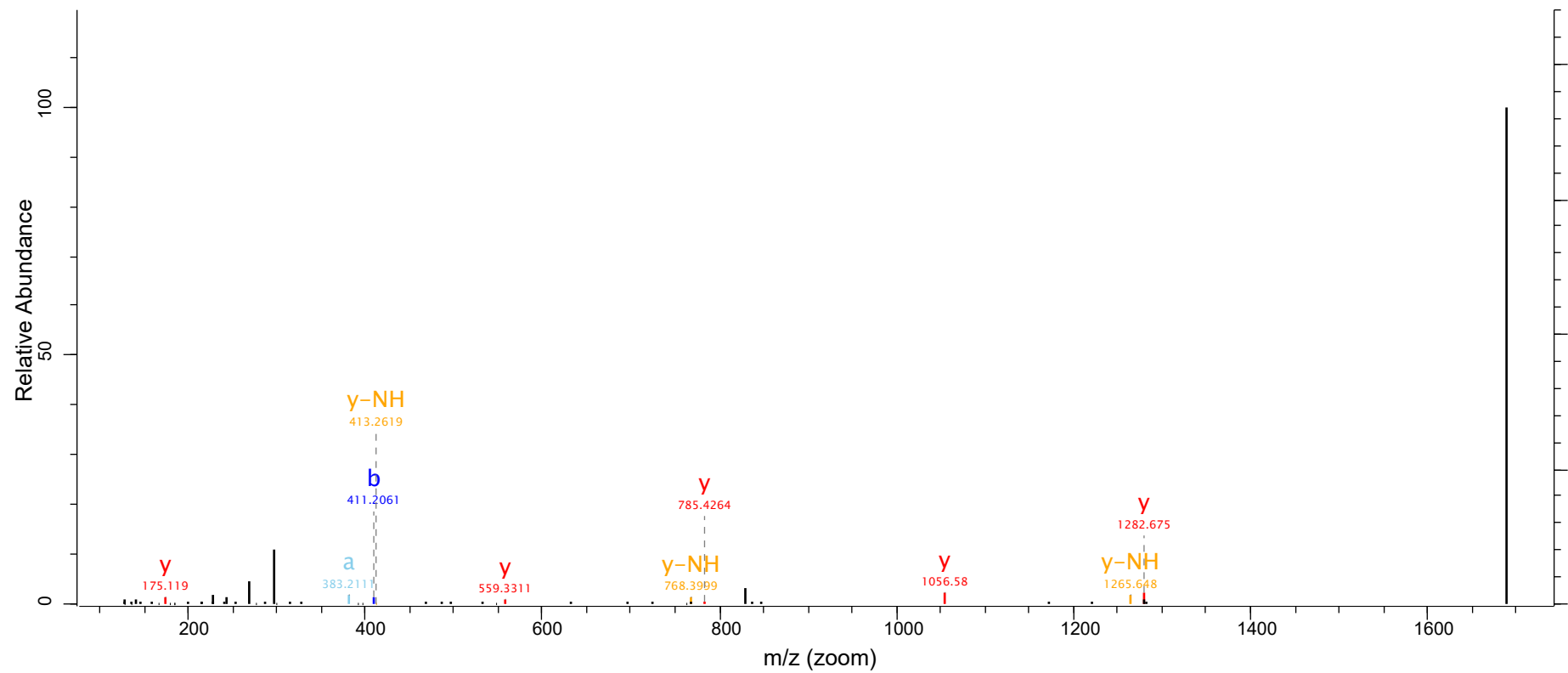
Scan
19070

Method
FTMS; HCD

Score
66.43

m/z
846.94

Gene names
ACSS2



Raw File
agg_pep_sort_pos_2

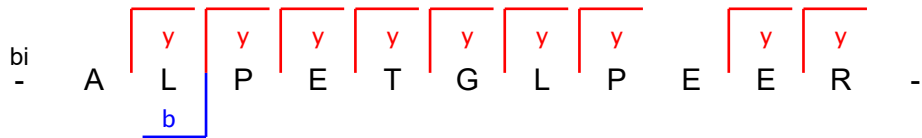
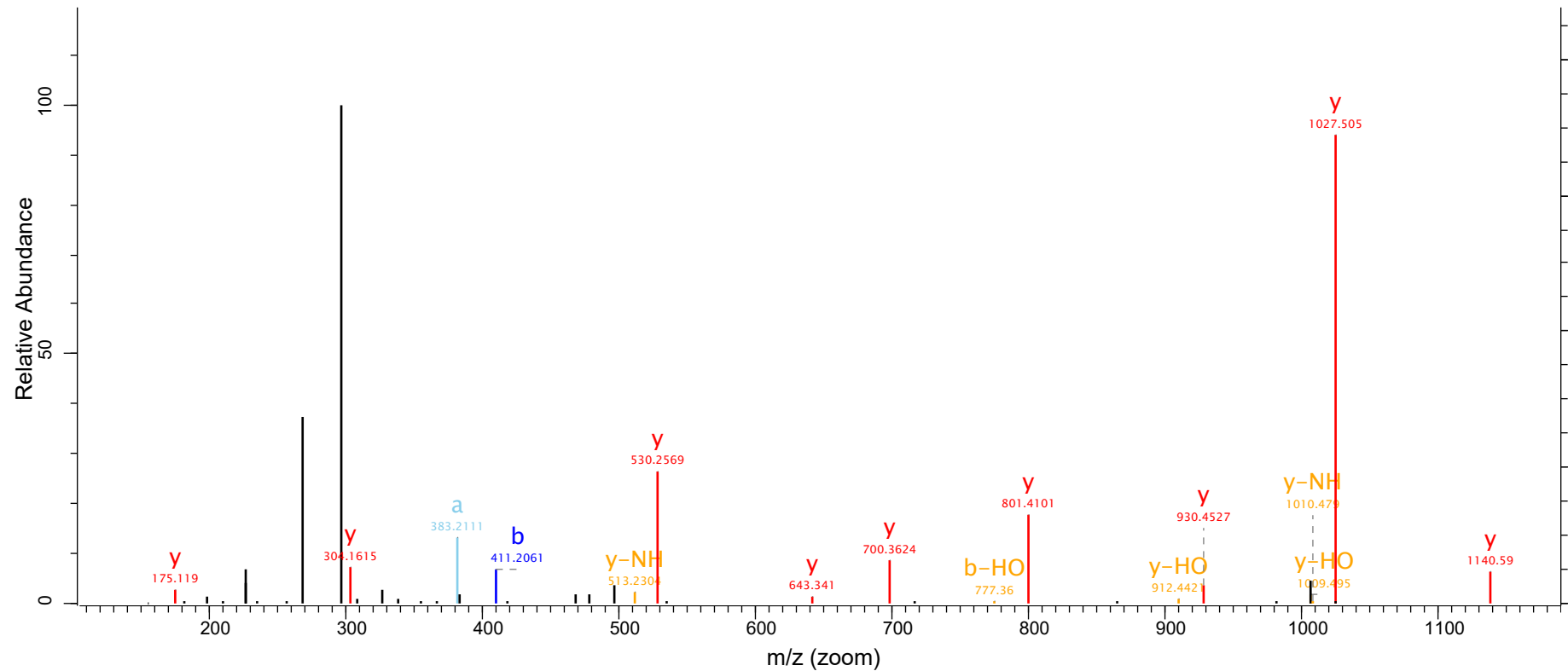
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21799

Method
FTMS; HCD

Score
105.4

m/z
719.36

Gene names
ACSS2



Raw File
agg_pep_sort_pos_3

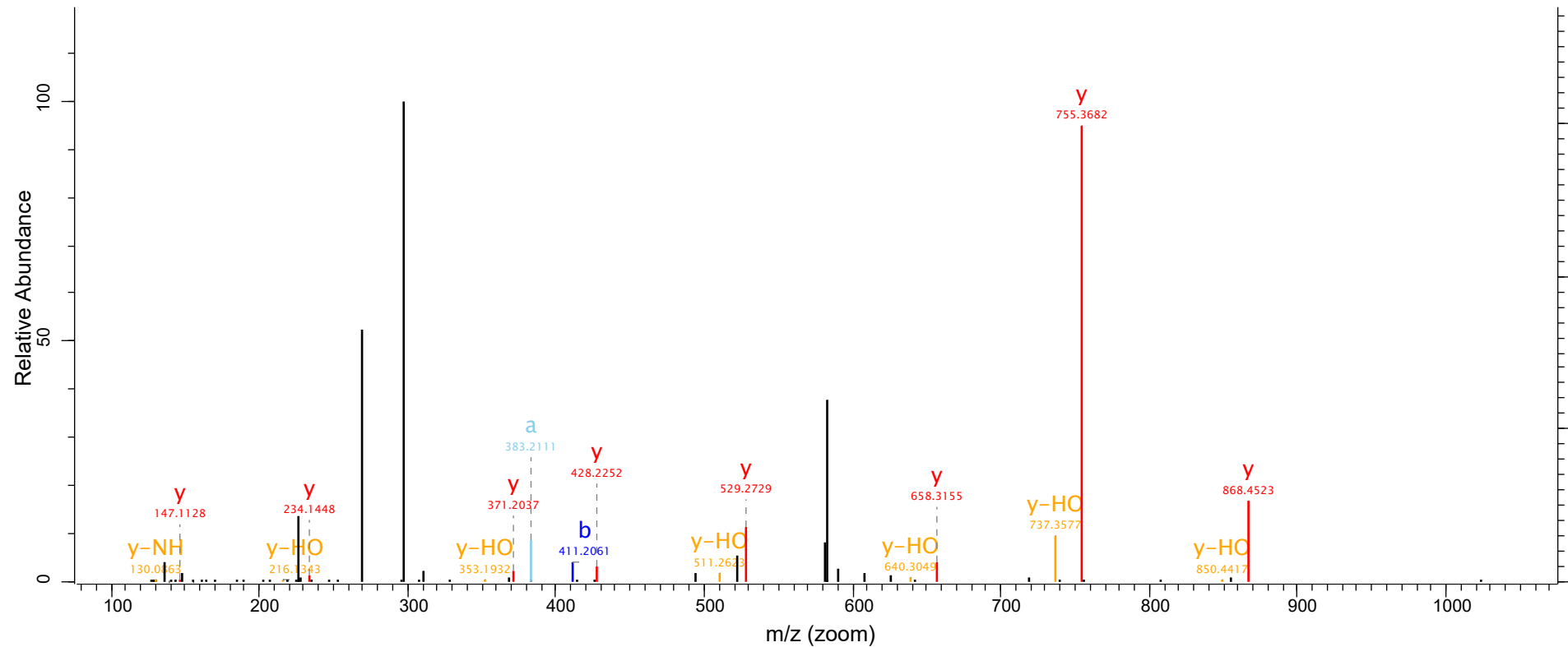
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11600

Method
FTMS; HCD

Score
102.51

m/z
583.29

Gene names
AGL



Raw File
agg_pep_sort_pos_2

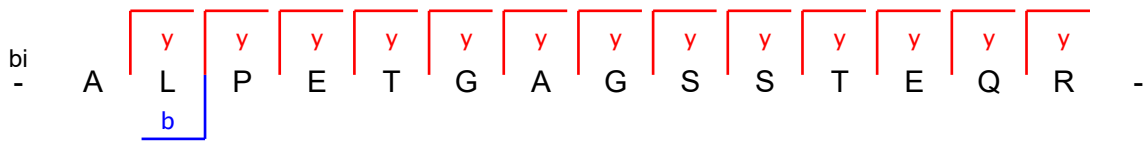
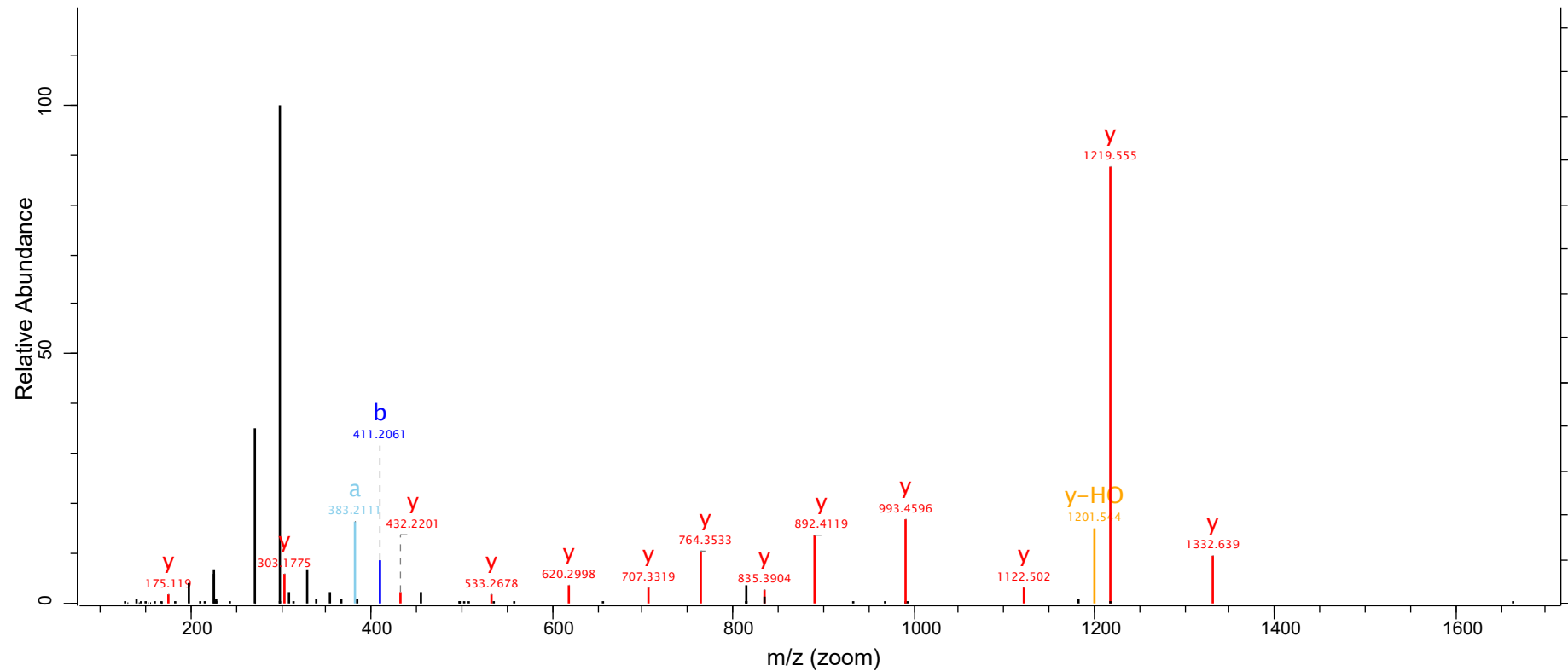
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Method
FTMS; HCD

Score
109.24

m/z
815.88

Gene names
AKAP12



Raw File
agg_pep_sort_pos_1

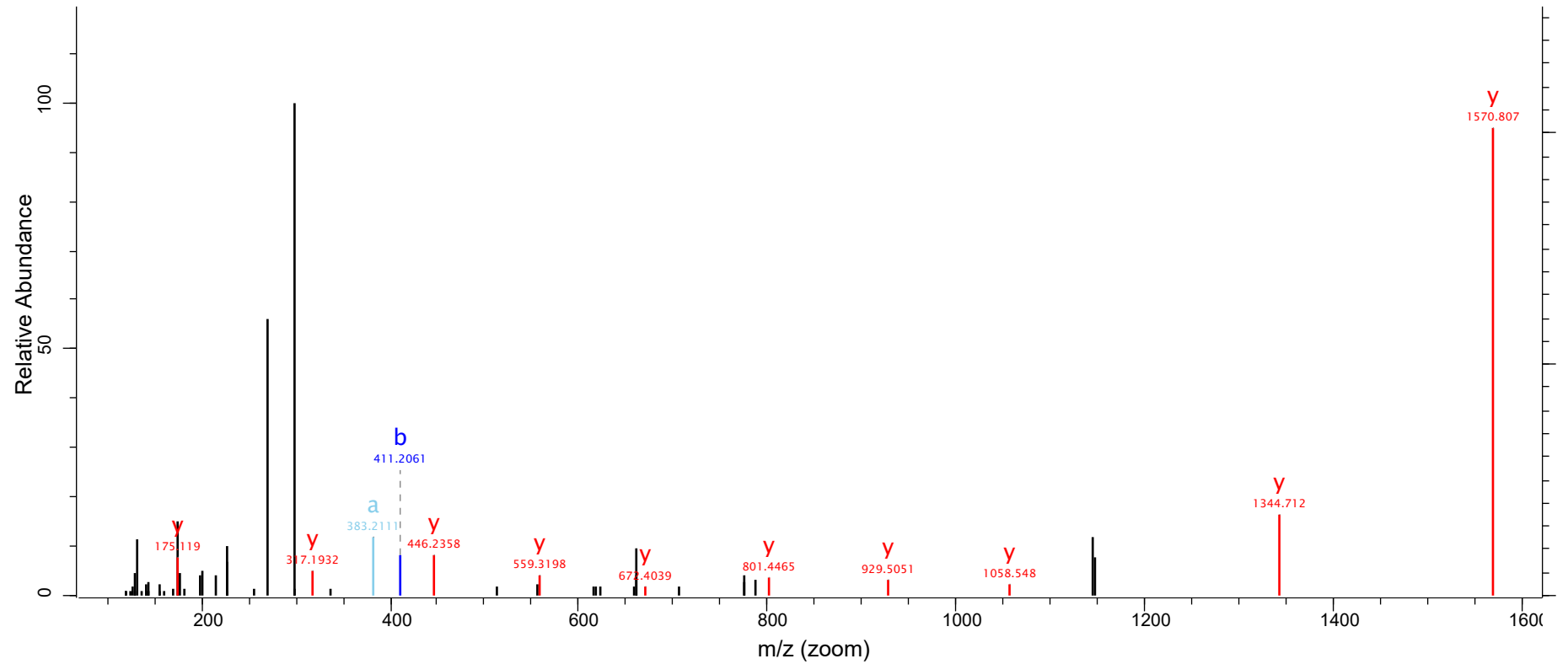
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20649

Method
FTMS; HCD

Score
52.52

m/z
661.34

Gene names
ANKS1A



bi
- bi
A L P E T G K E Q E L L E A A R -

Fragmentation diagram showing peptide sequence: A L P E T G K E Q E L L E A A R. Red brackets above the sequence indicate b and y ion fragments. A blue bracket below the sequence indicates a b ion fragment.

Raw File
agg_pep_sort_pos_1

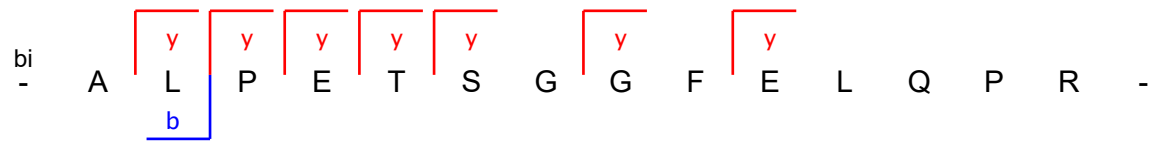
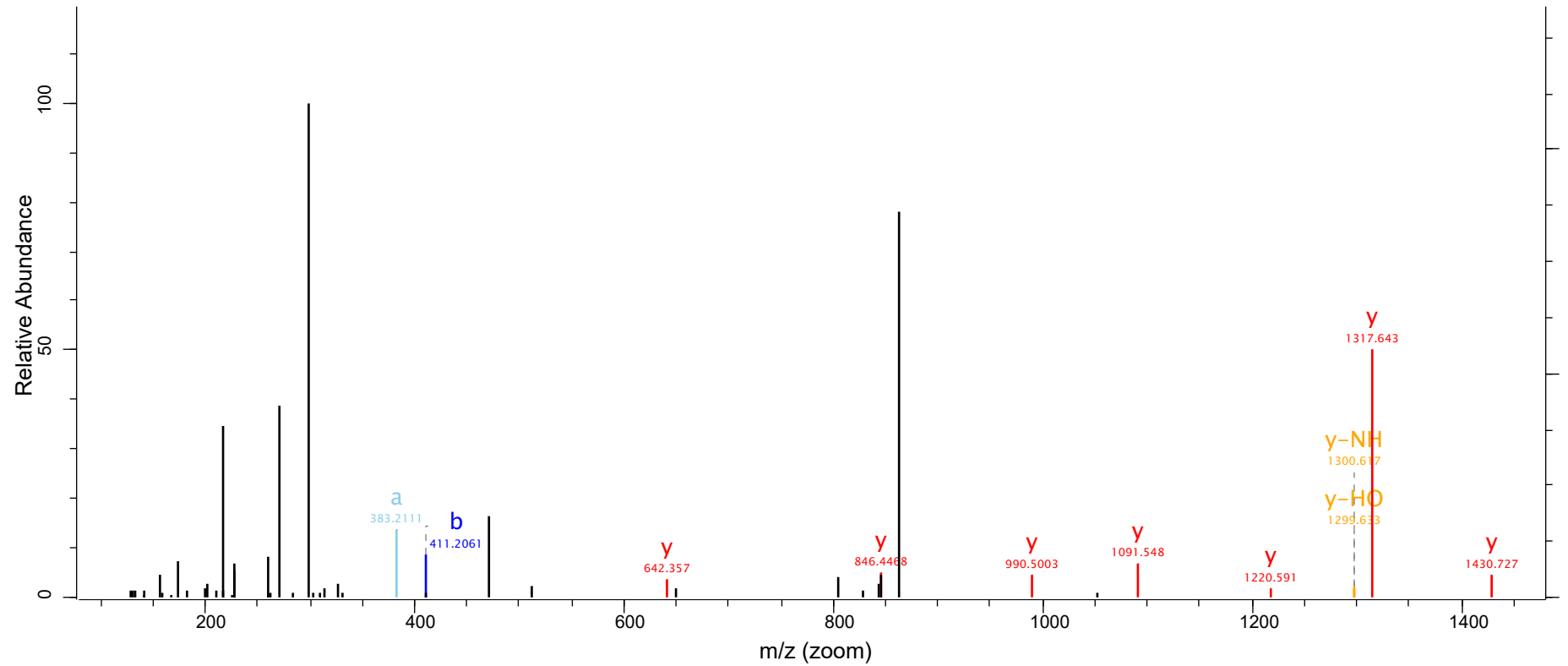
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22174

Method
FTMS; HCD

Score
56.36

m/z
864.42

Gene names
APLF



Raw File
agg_pep_sort_pos_1

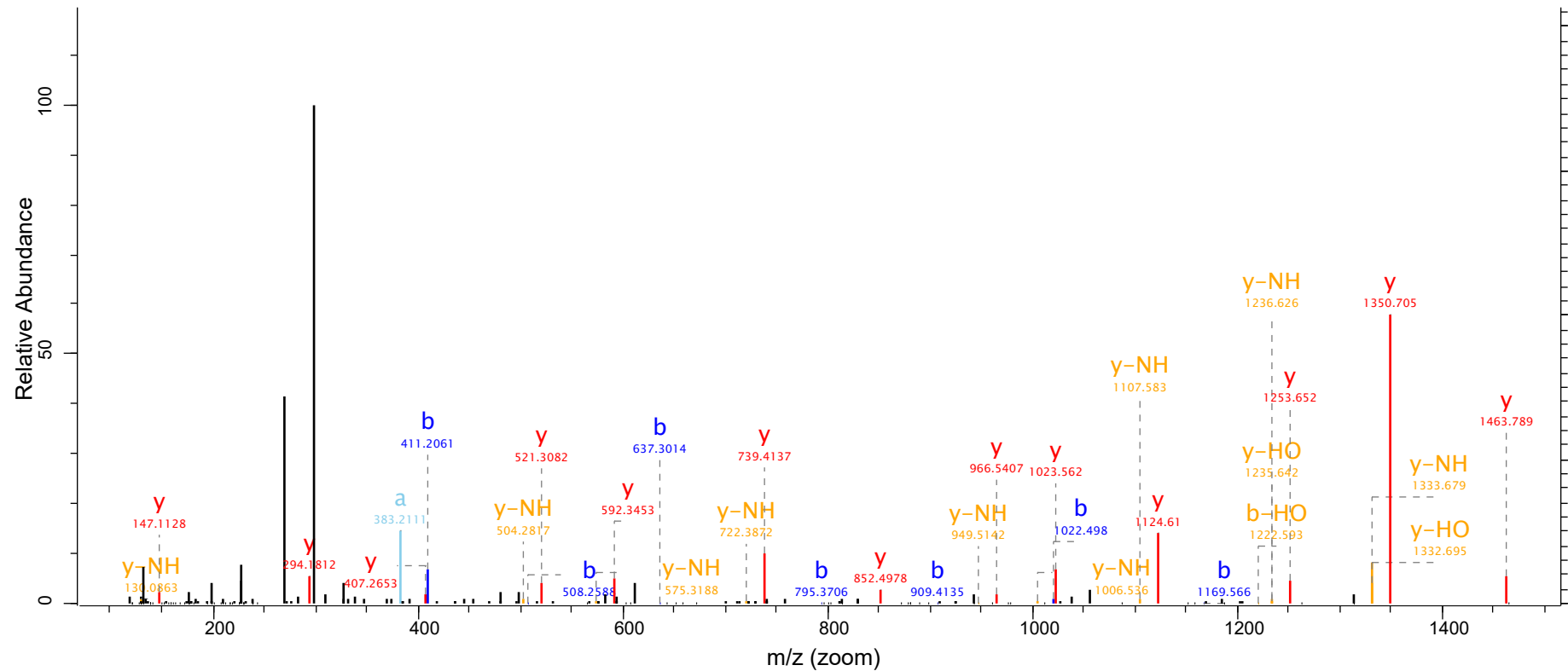
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31832

Method
FTMS; HCD

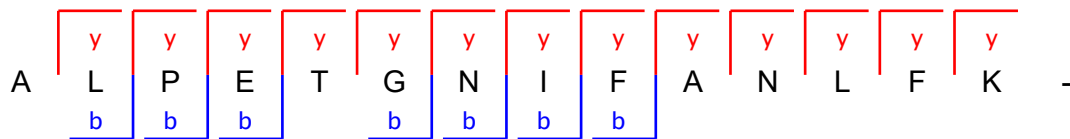
Score
168.69

m/z
880.96

Gene names
ARF1



bi



Raw File
agg_pep_sort_pos_1

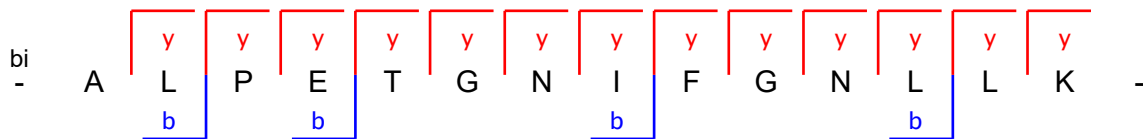
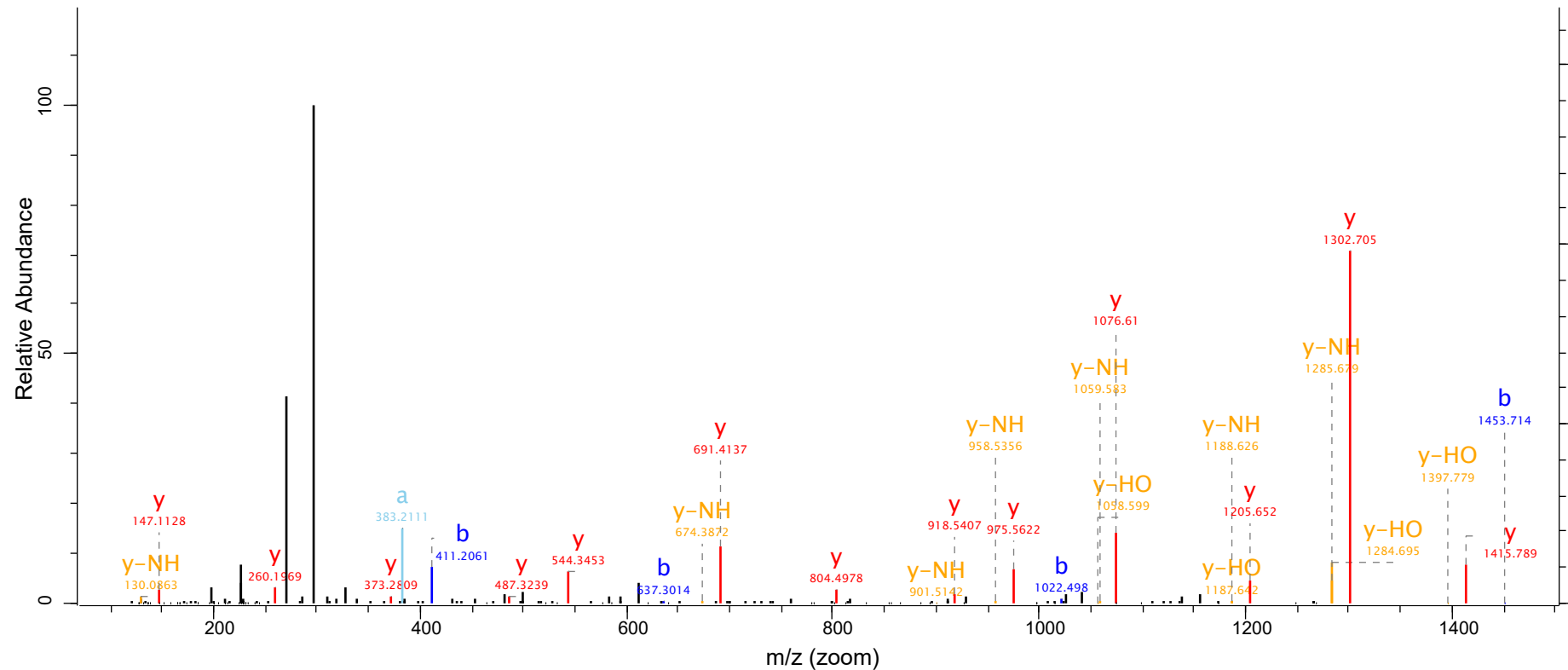
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31640

Method
FTMS; HCD

Score
190.86

m/z
857.46

Gene names
ARF3



Raw File
agg_pep_sort_pos_1

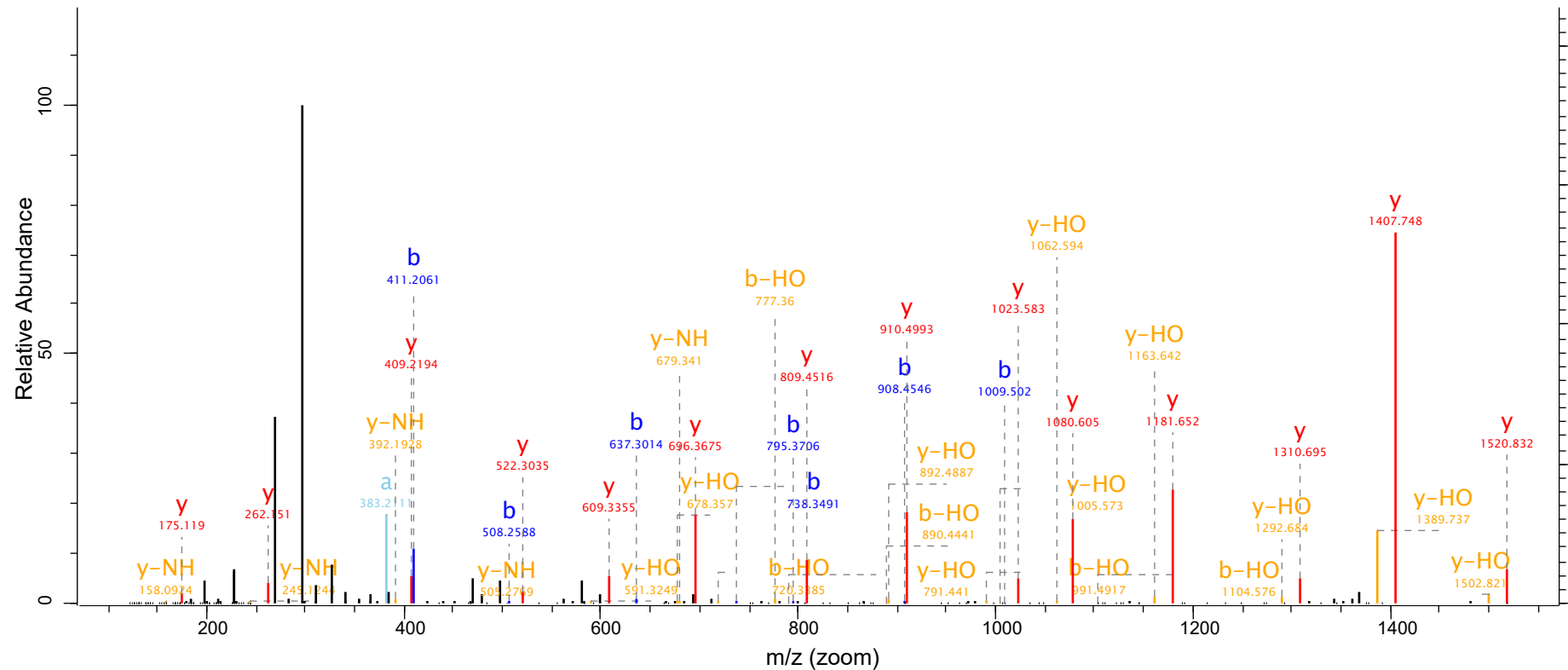
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31721

Method
FTMS; HCD

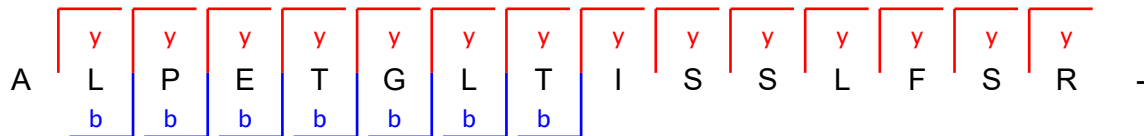
Score
250.26

m/z
909.48

Gene names
ARF4



- bi



Raw File
agg_pep_sort_pos_3

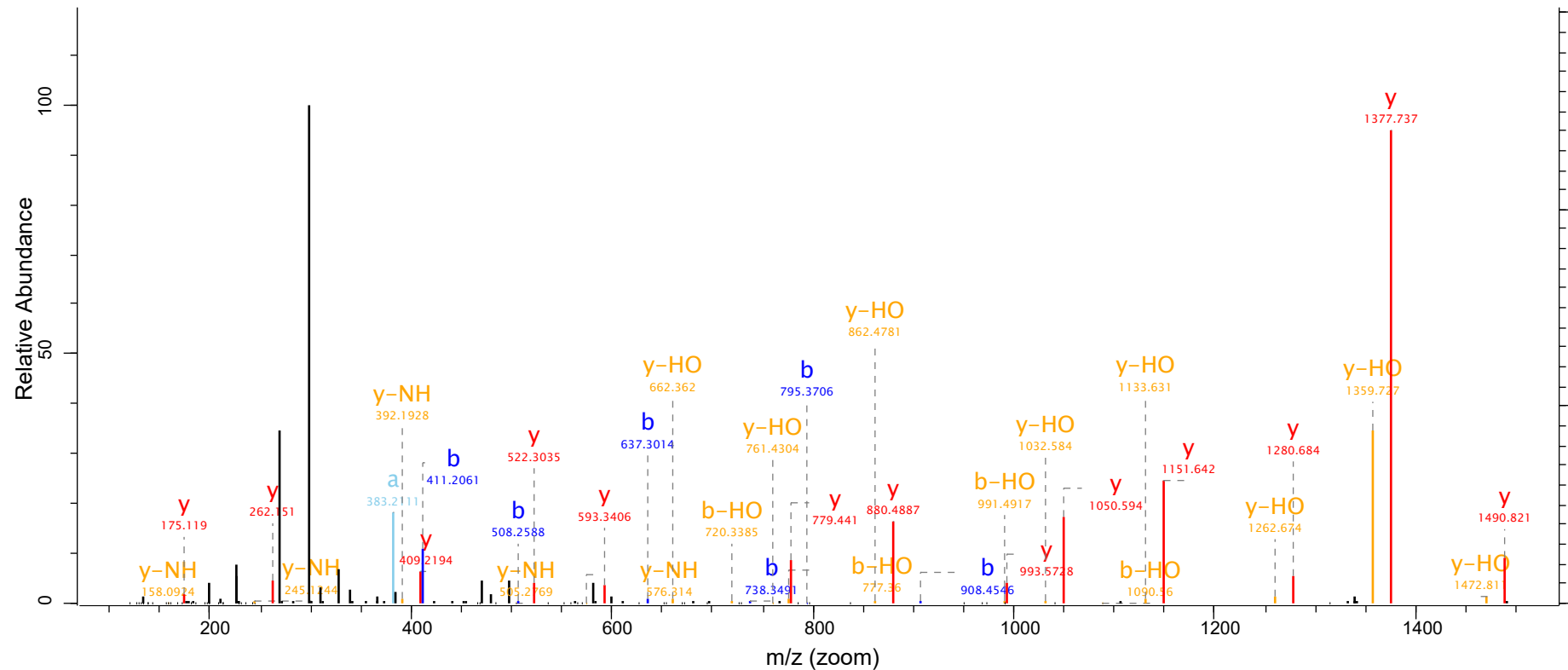
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31282

Method
FTMS; HCD

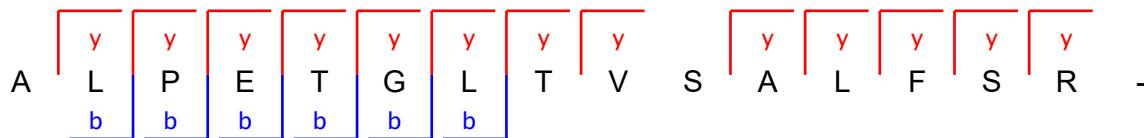
Score
207.88

m/z
894.47

Gene names
ARF5



- bi



Raw File
agg_pep_sort_pos_3

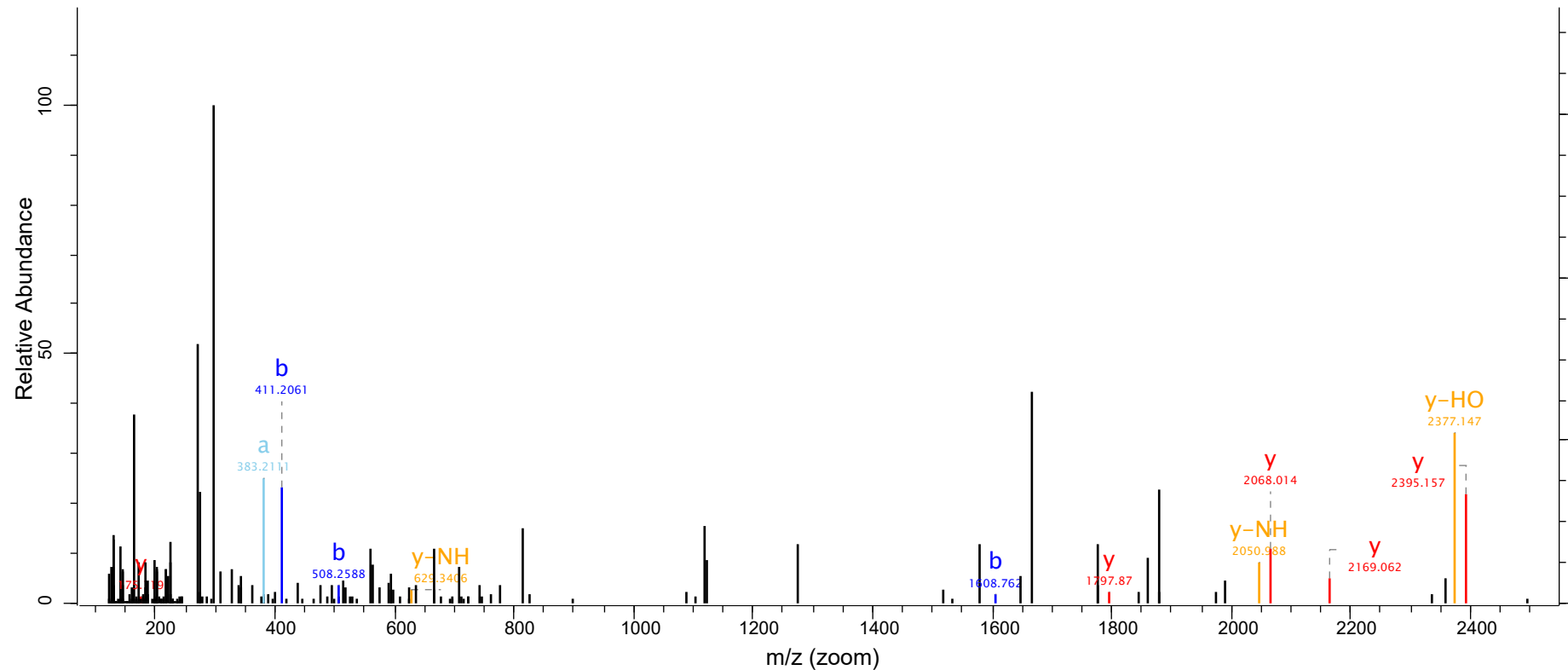
Scan
7498

Method
FTMS; HCD

Score
27.9

m/z
562.08

Gene names
ARGFX



bi

A L P E T R N R M^{ox} A P E N P Q P D P F I N R -

Fragmentation diagram showing b-ions (blue) and y-ions (red) for the peptide sequence: A L P E T R N R M^{ox} A P E N P Q P D P F I N R -.

Fragmentation sites are indicated by brackets below the sequence:

- Red brackets (y-ions) connect P to E, T to R, R to N, and N to R.
- Blue brackets (b-ions) connect L to P, P to E, and E to N.

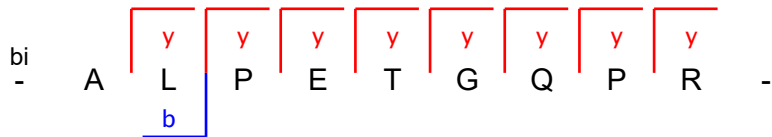
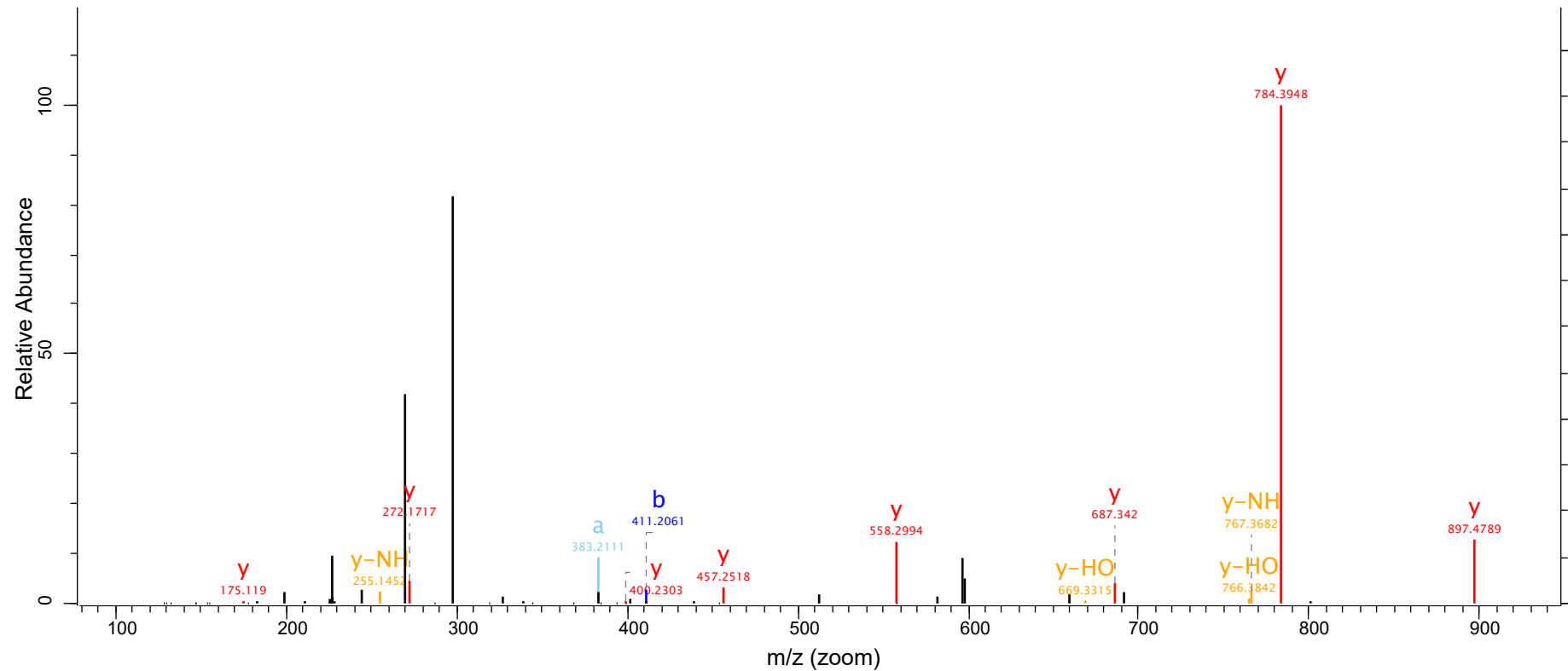
Raw File
agg_pep_sort_pos_2

Scan
17275

Method
FTMS; HCD

Score
98.52

m/z
597.8



Raw File
agg_pep_sort_pos_1

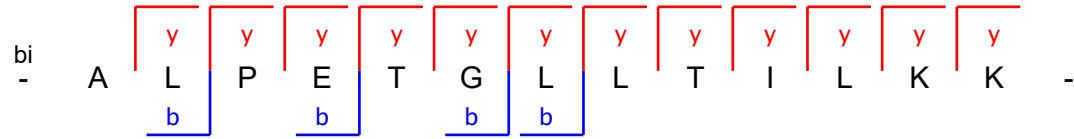
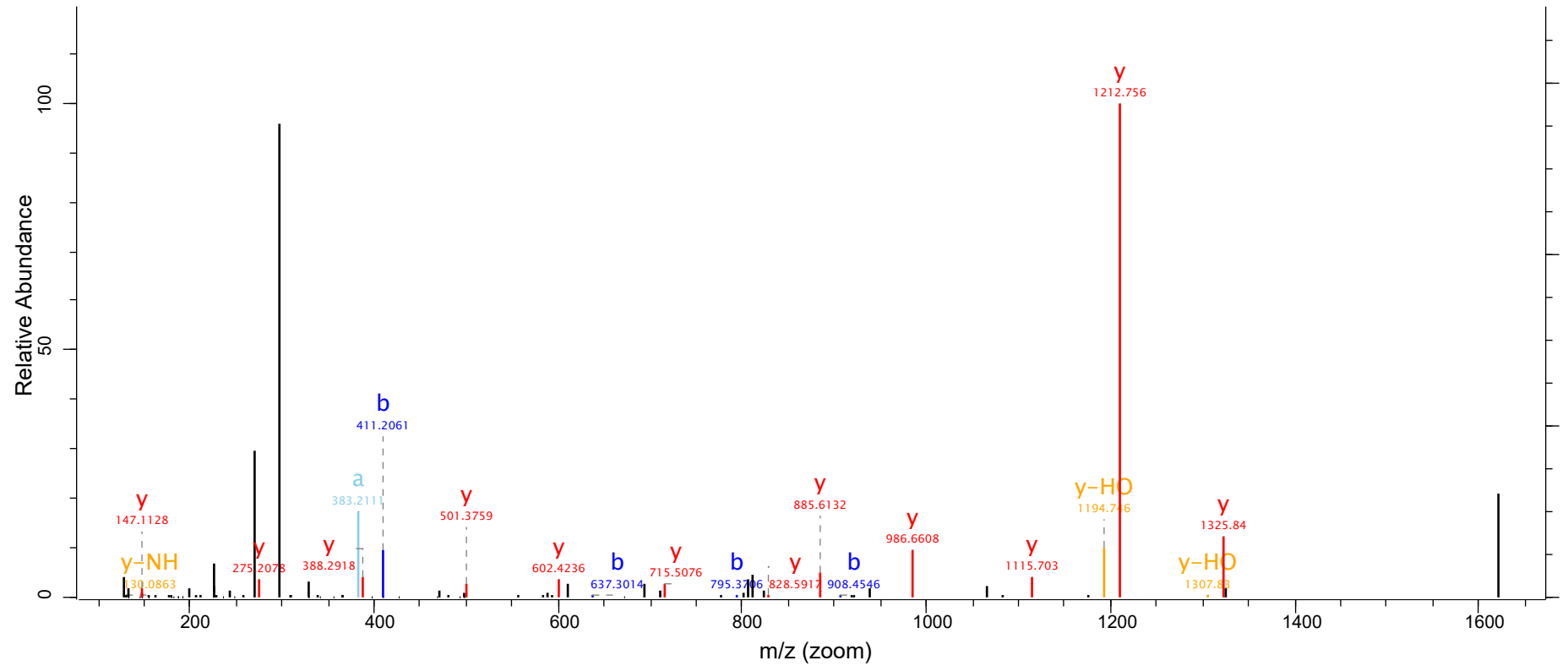
Scan
26814

Method
FTMS; HCD

Score
107.06

m/z
811.98

Gene names
ARL2



Raw File
agg_pep_sort_pos_3

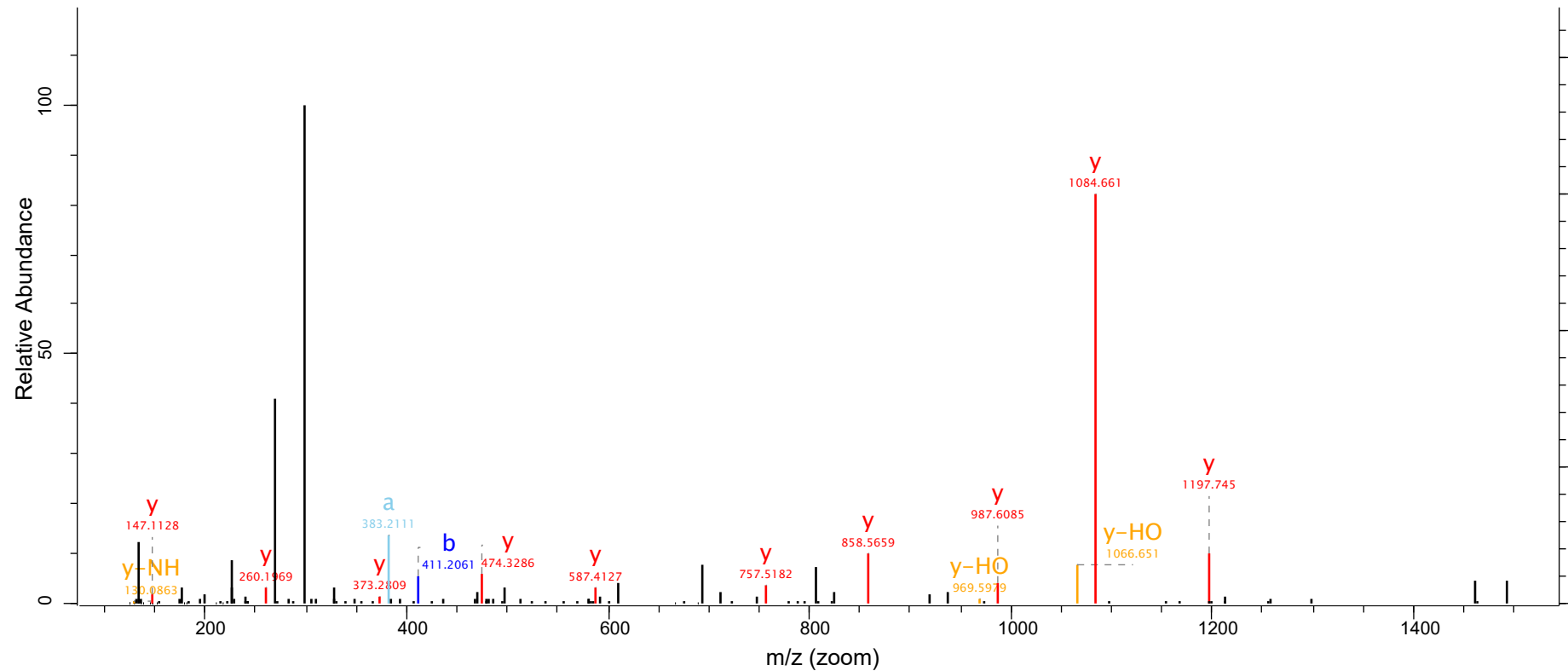
Scan
31323

Method
FTMS; HCD

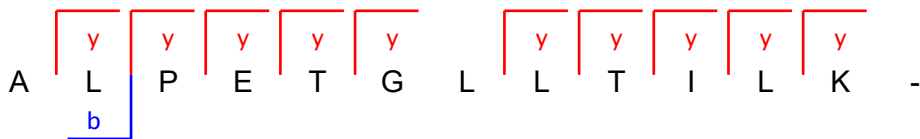
Score
98.05

m/z
747.94

Gene names
ARL2



bi



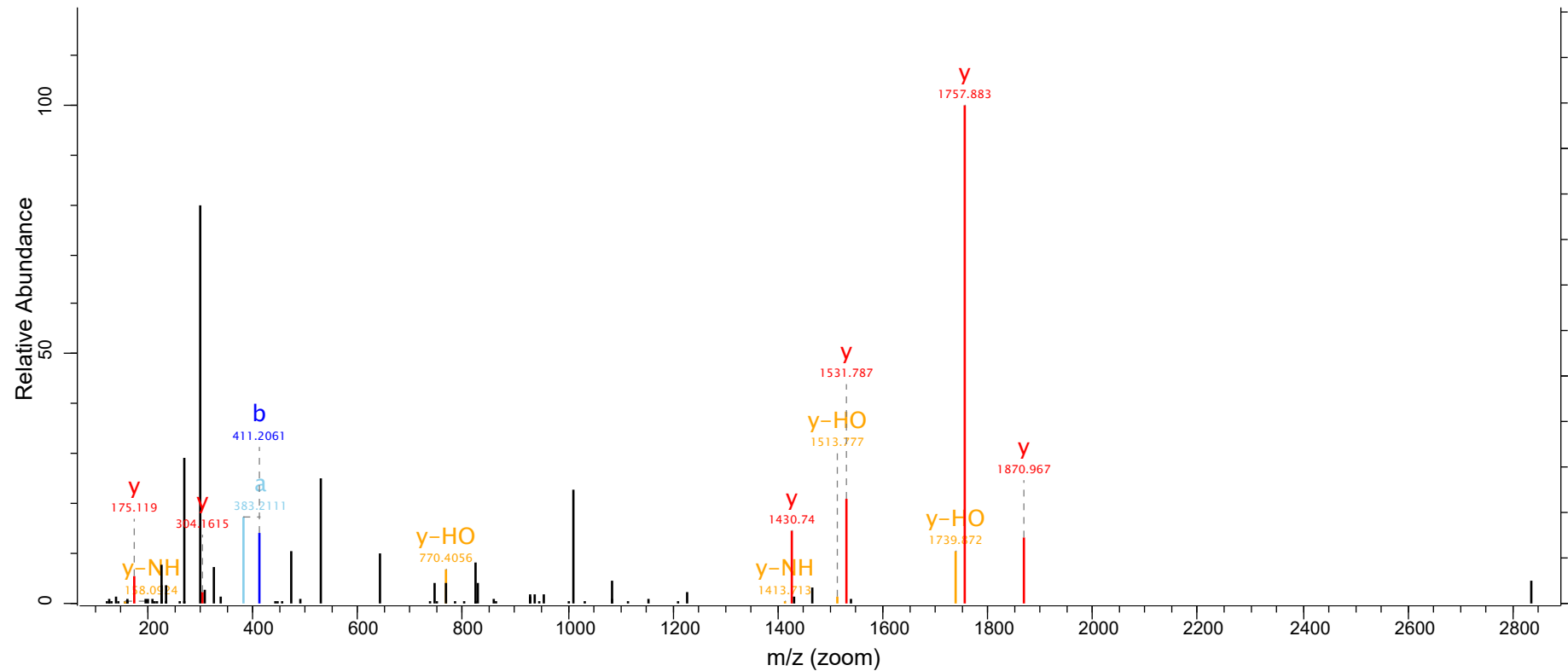
Raw File
agg_pep_sort_pos_1

Scan
18578

Method
FTMS; HCD

Score
68.07

m/z
1085.04



bi



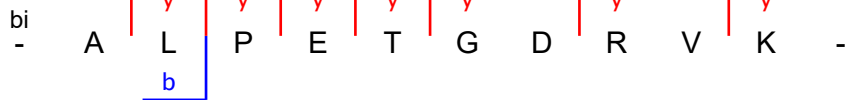
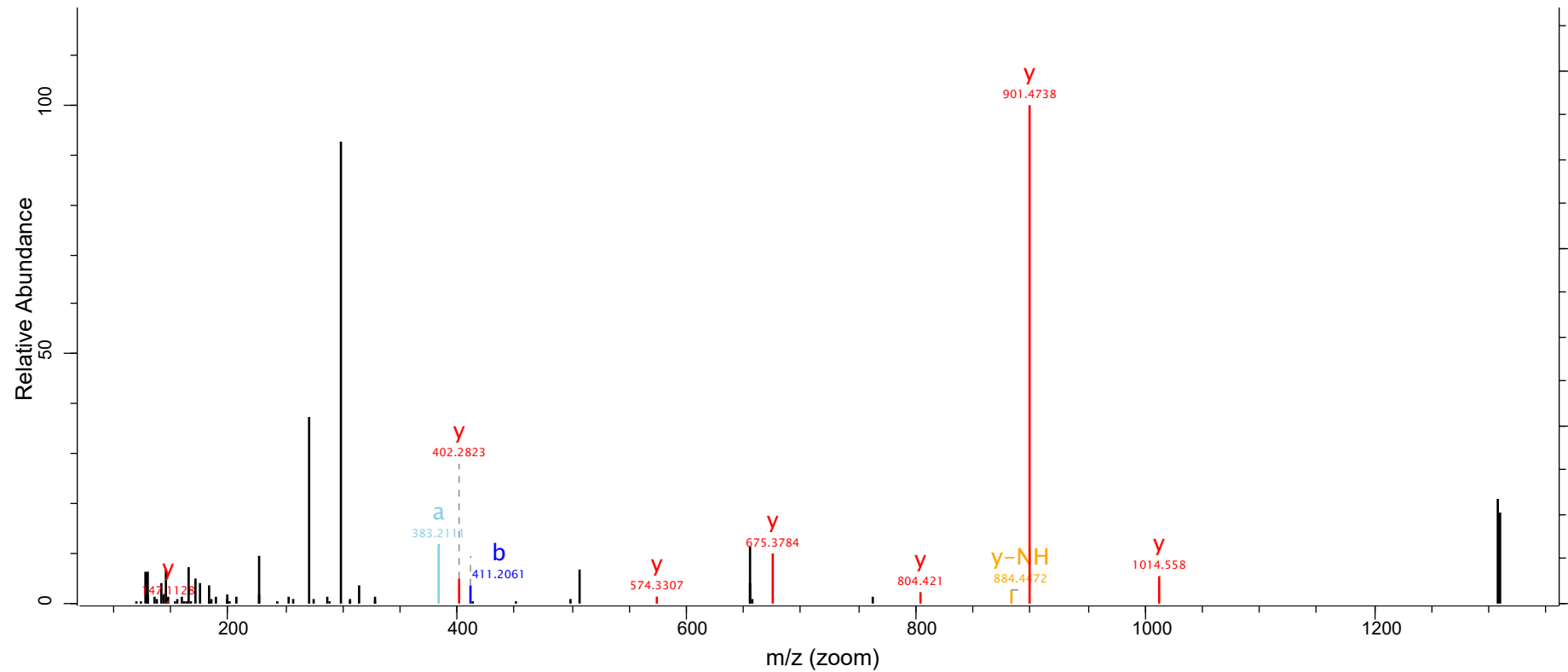
Raw File
agg_pep_sort_pos_1

Scan
14207

Method
FTMS; HCD

Score
67.99

m/z
656.34



Raw File
agg_pep_sort_pos_1

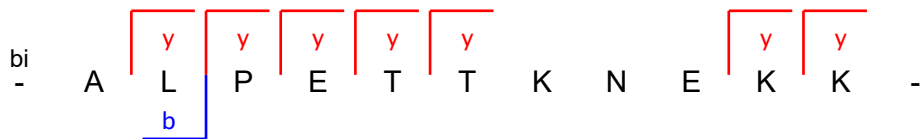
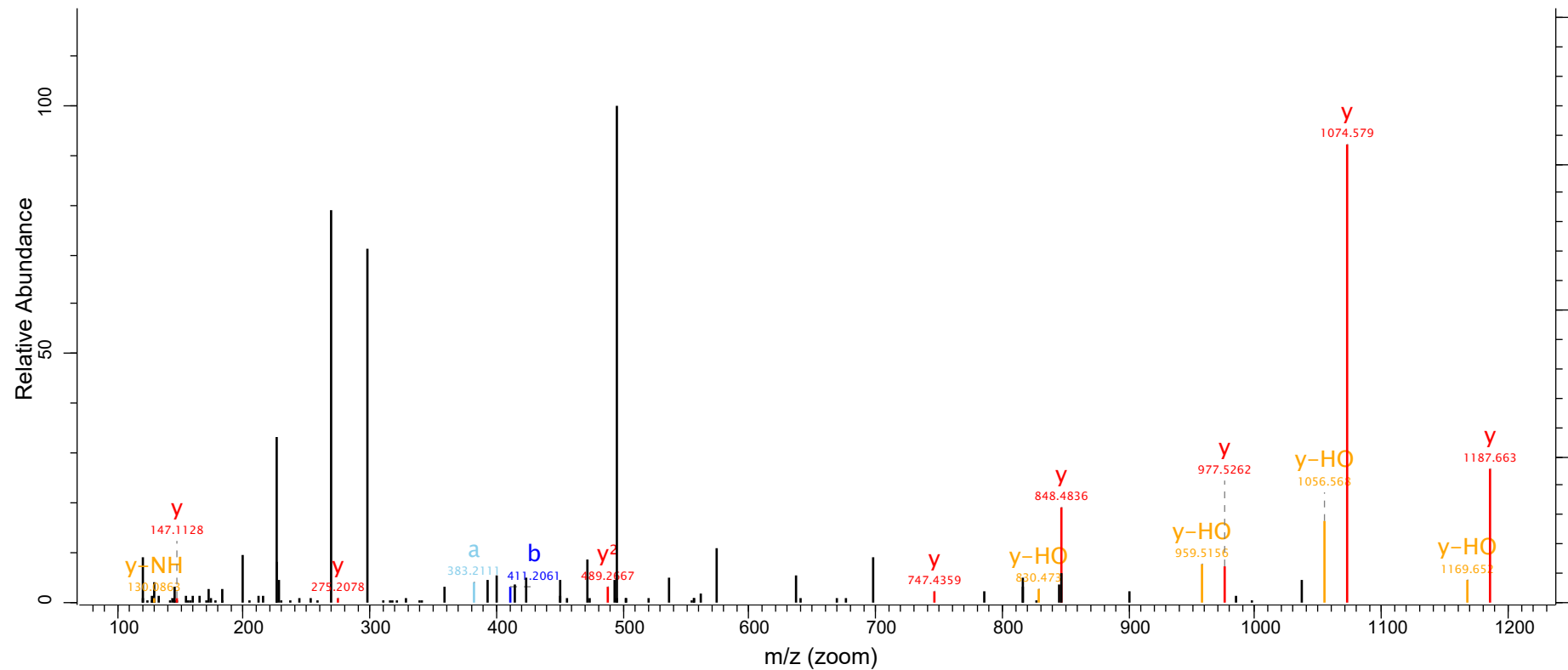
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9512

Method
FTMS; HCD

Score
57.84

m/z
495.6

Gene names
ATP1B3



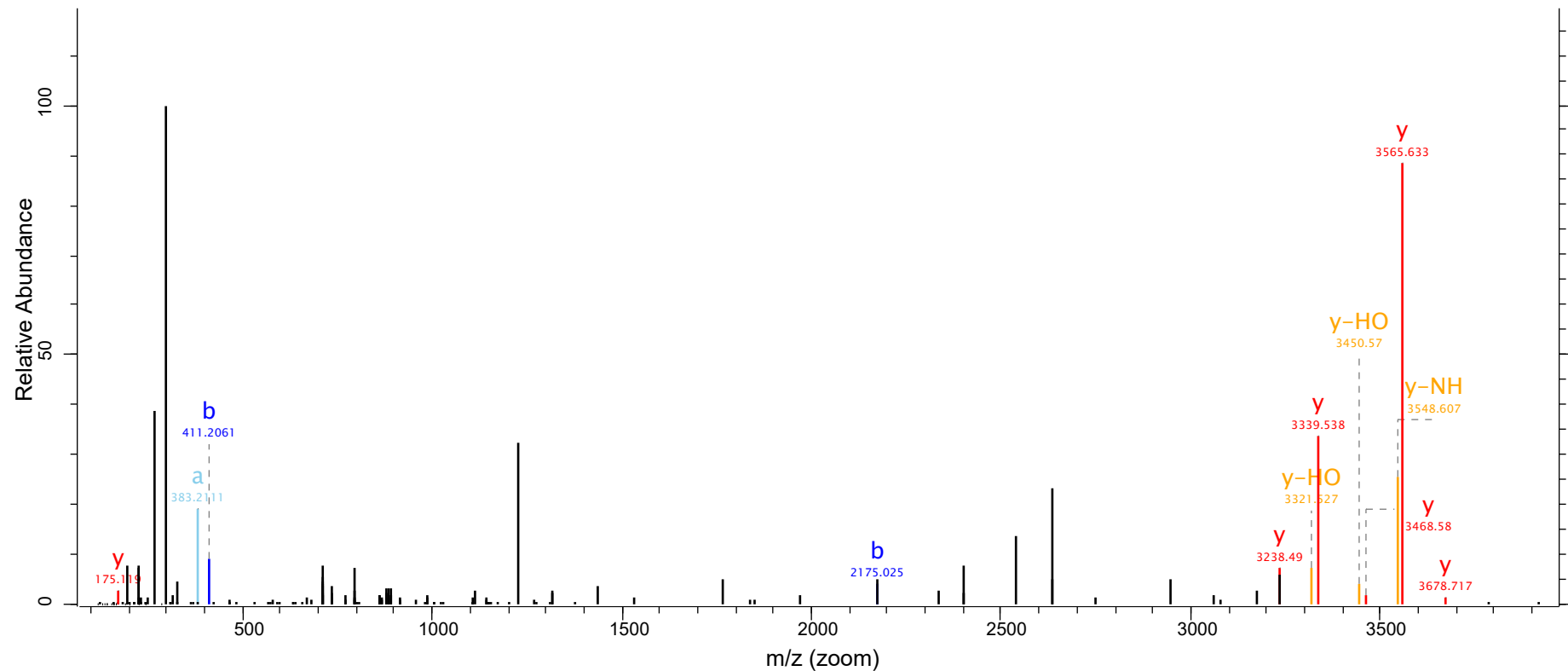
Raw File
agg_pep_sort_pos_1

Scan
17652

Method
FTMS; HCD

Score
38.03

m/z
796.37



bi - A L P E T A V E V Q E Q W P C L P A A G C P G P
L G G P V A A C G M^{ox} S R -

Fragmentation diagram showing peptide sequences and fragmentation sites:

- Top sequence: A L P E T A V E V Q E Q W P C L P A A G C P G P
- Bottom sequence: L G G P V A A C G M^{ox} S R -
- Red brackets indicate y-ions: L, P, E, T, A, P, A, G, C, P
- Blue brackets indicate b-ions: L, P, P

Raw File
agg_pep_sort_pos_1

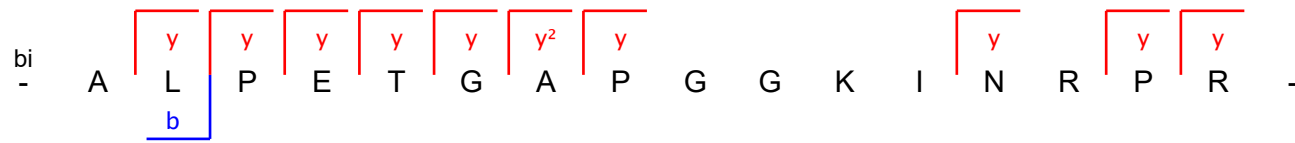
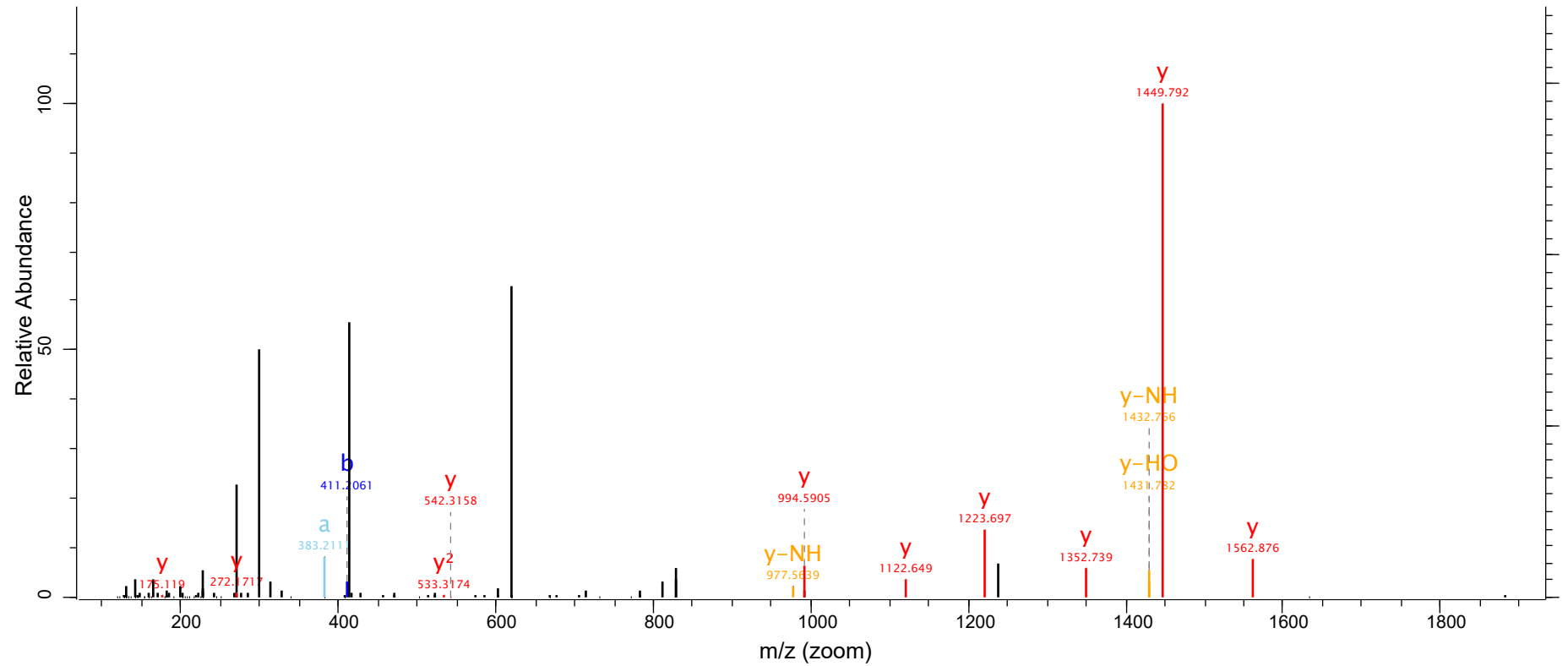
Scan
12771

Method
FTMS; HCD

Score
47.55

m/z
621

Gene names
C11orf98



Raw File
agg_pep_sort_pos_1

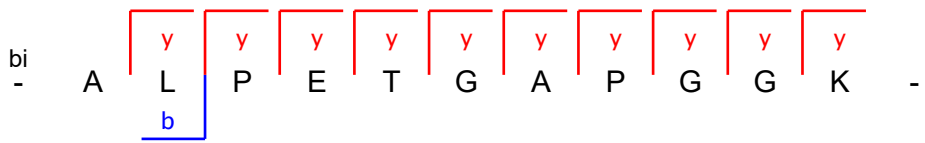
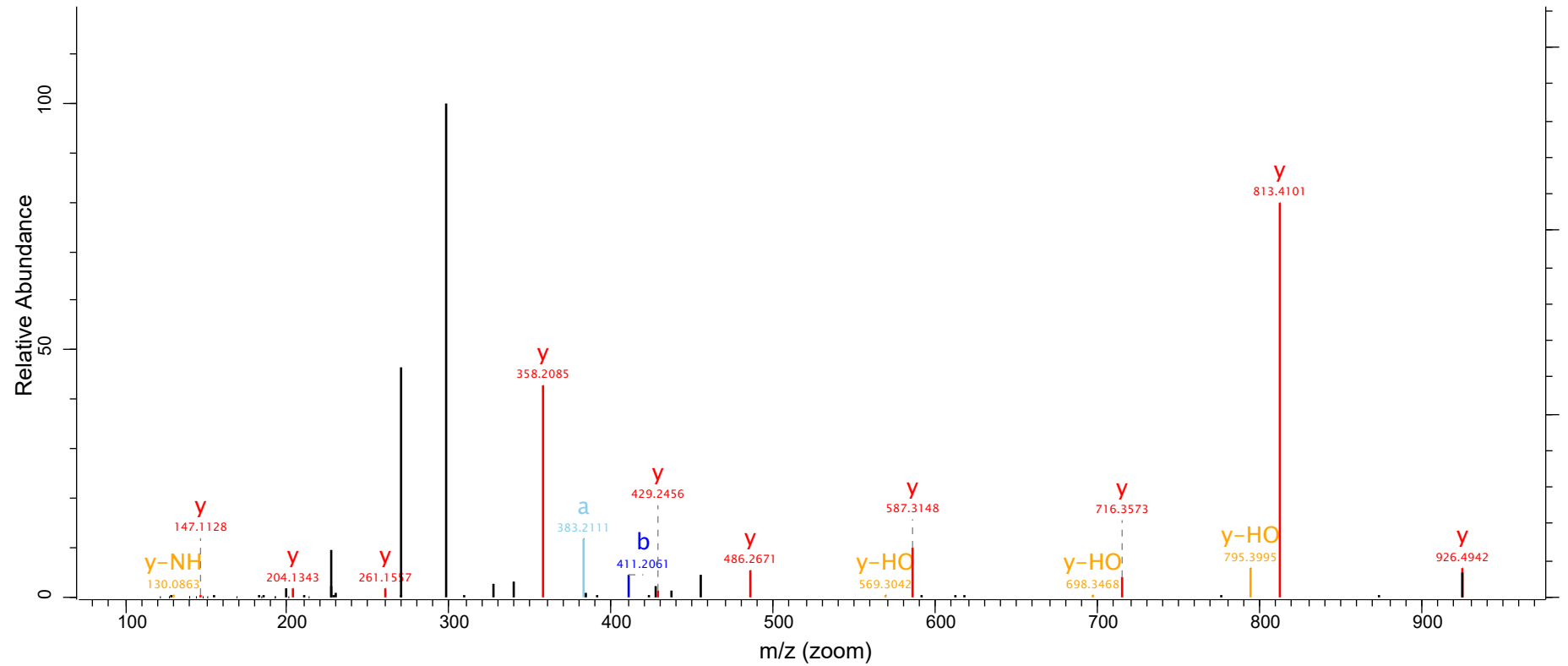
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17941

Method
FTMS; HCD

Score
95.5

m/z
612.31

Gene names
C11orf98



Raw File
agg_pep_sort_pos_3

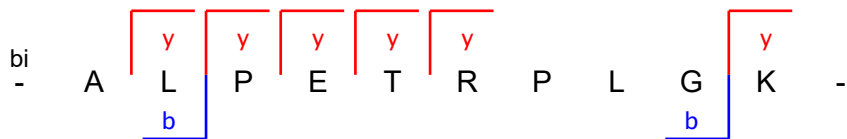
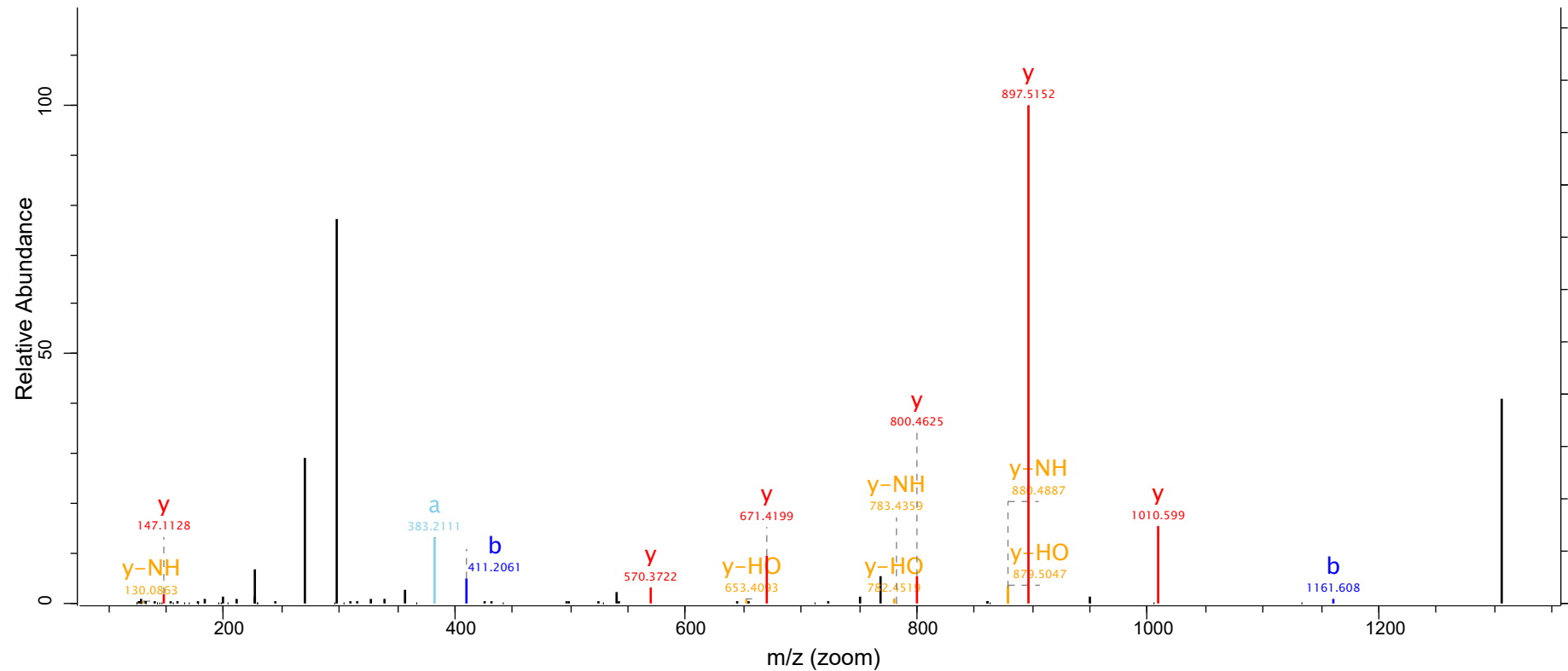
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Method
FTMS; HCD

Score
111.04

m/z
654.36

Gene names
C8orf86



Raw File
agg_pep_sort_pos_3

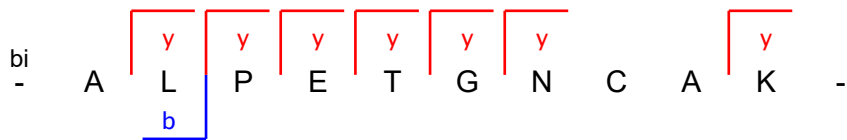
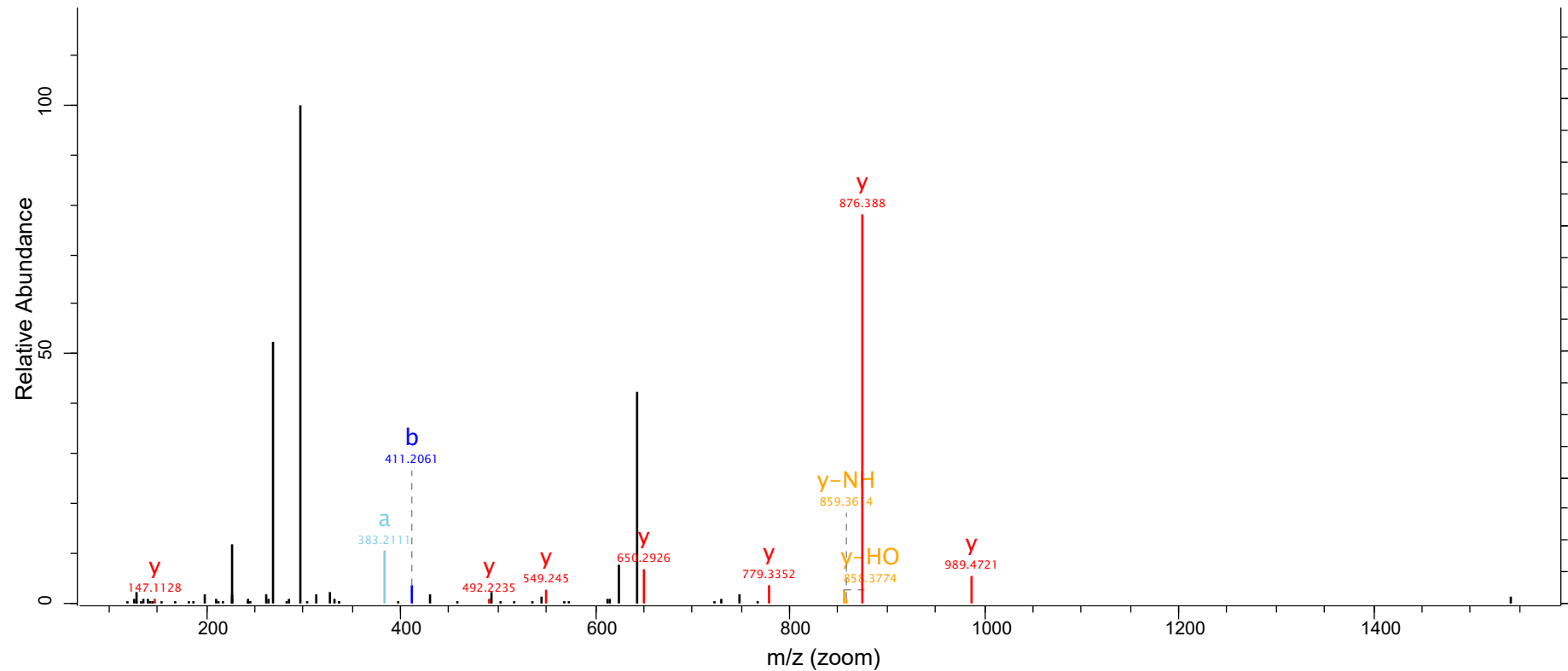
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Method
FTMS; HCD

Score
94.3

m/z
643.8

Gene names
CABP2



Raw File
agg_pep_sort_pos_3

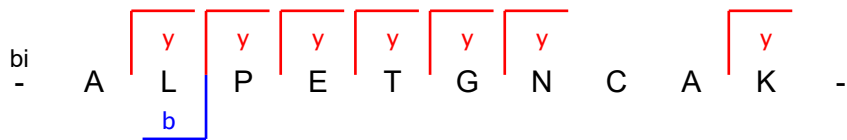
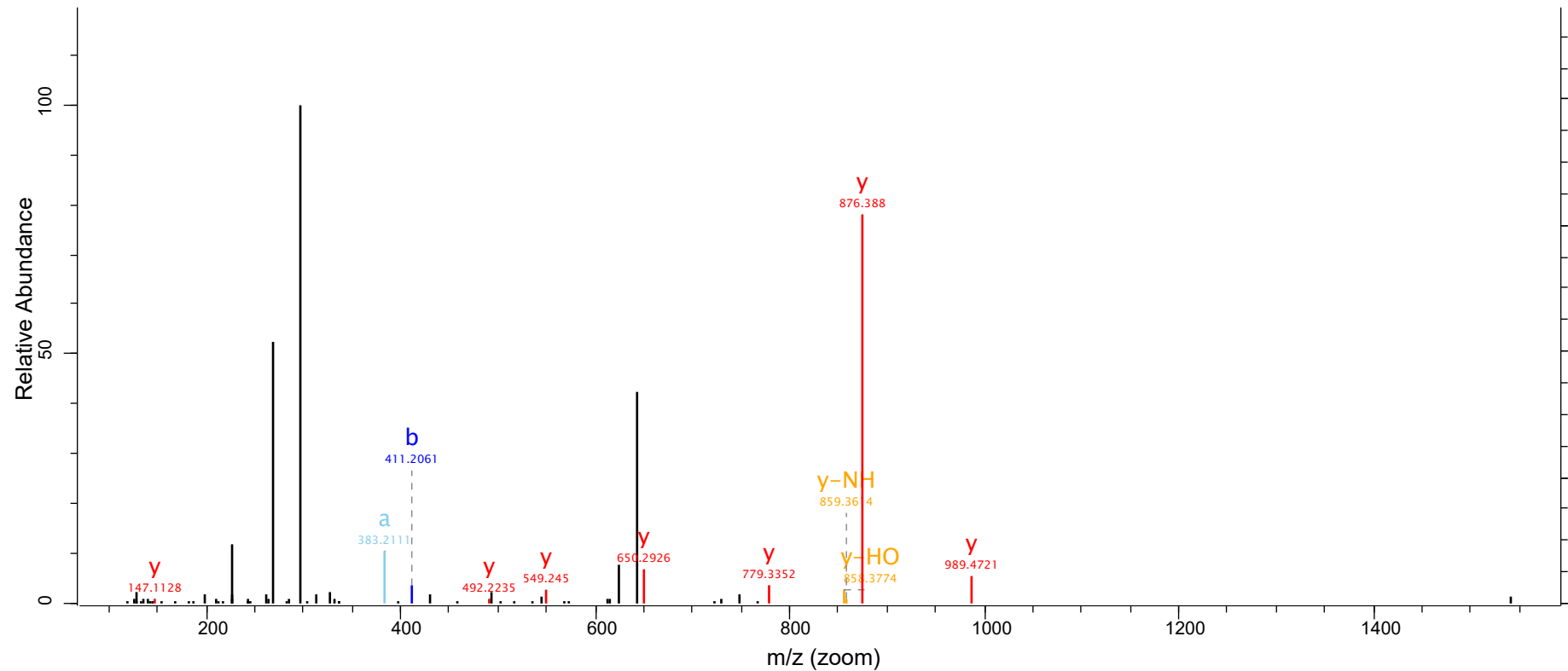
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16400

Method
FTMS; HCD

Score
94.3

m/z
643.8

Gene names
CABP2



Raw File
agg_pep_sort_pos_3

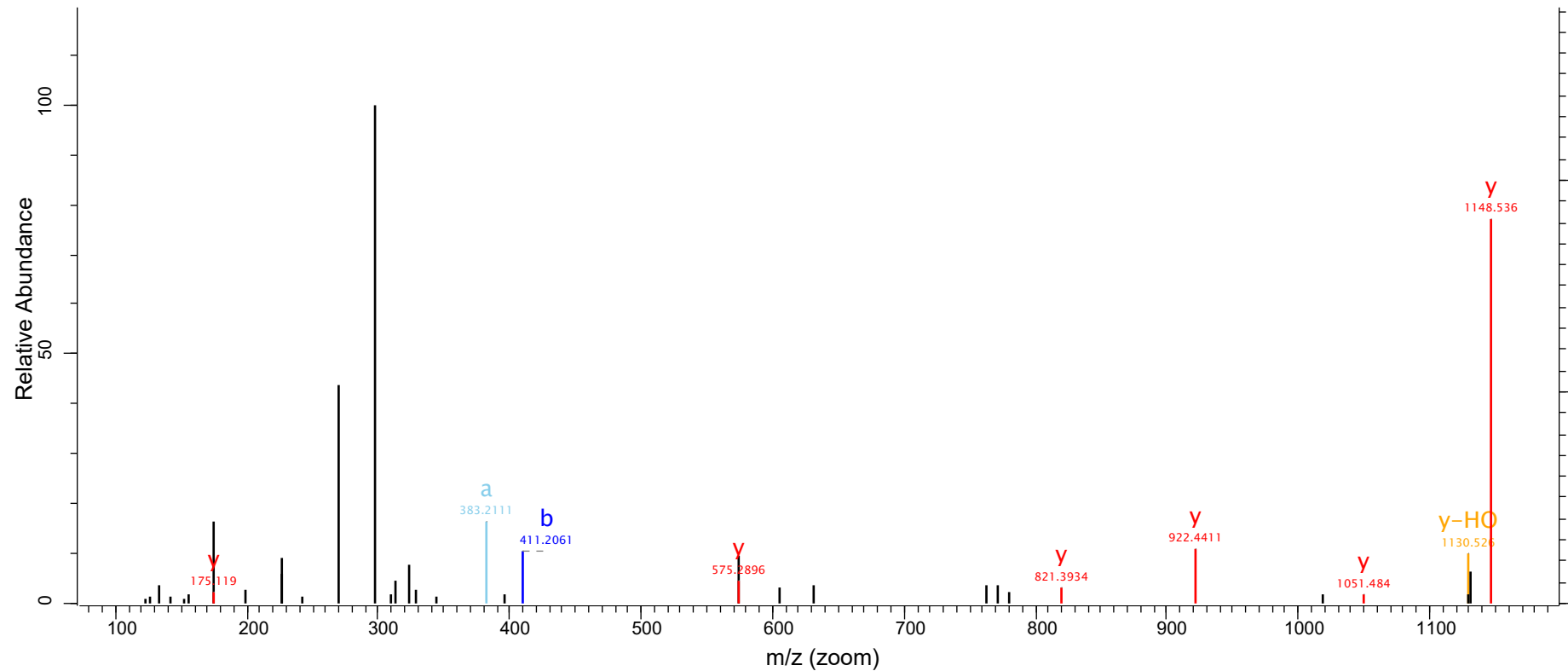
Scan
20007

Method
FTMS; HCD

Score
48.42

m/z
779.87

Gene names
CALU



bi - A L P E T M V R D E R -

Fragmentation diagram showing b and y ions. A blue 'b' ion is shown below the L residue. Red 'y' ions are shown above the P, E, T, M, R, and the C-terminal dash (-) residues. Oxidation (ox) is indicated above the M residue.

Raw File
agg_pep_sort_pos_2

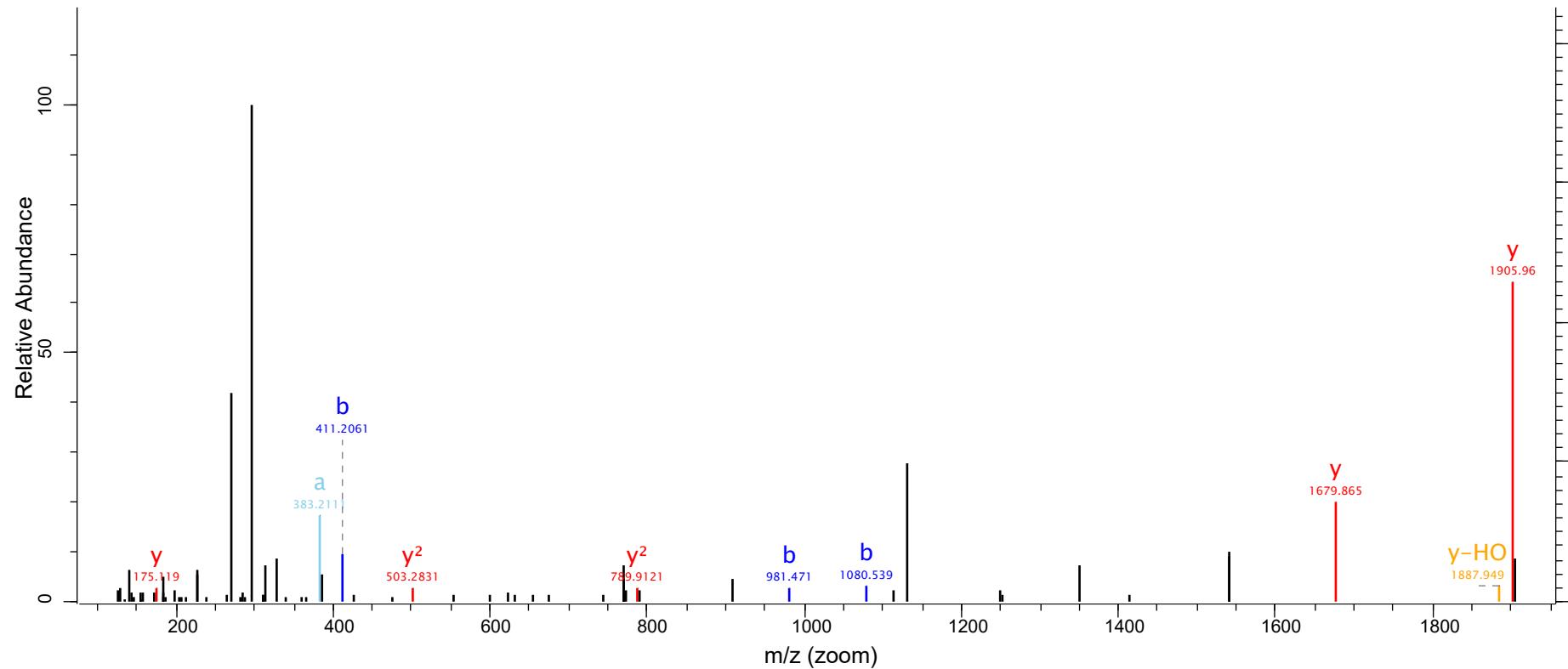
Scan
16537

Method
FTMS; HCD

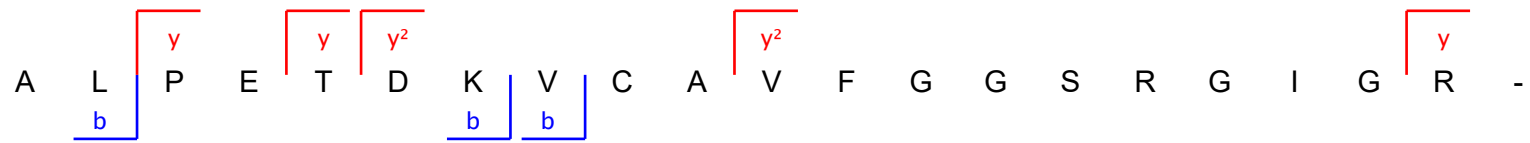
Score
40.84

m/z
773.06

Gene names
CBR4



bi



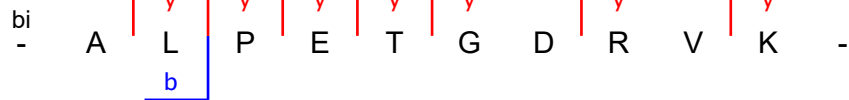
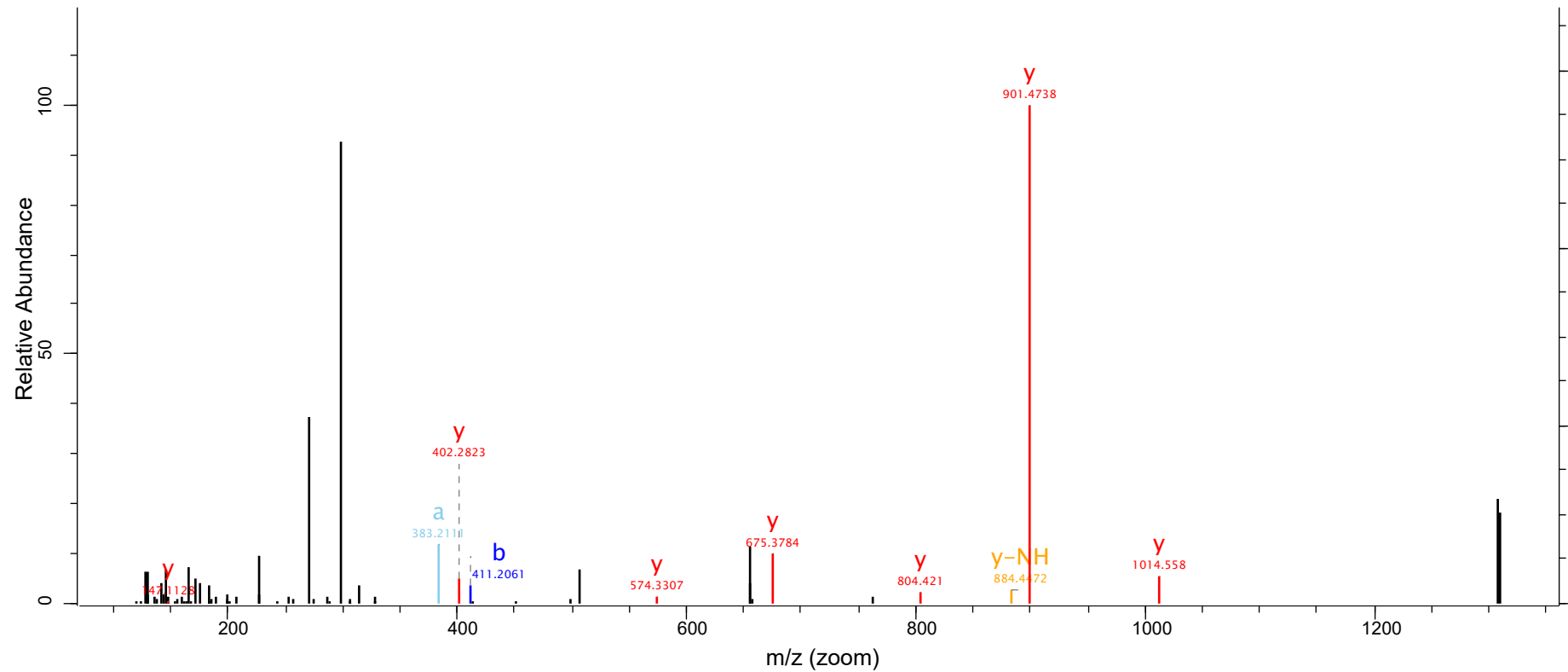
Raw File
agg_pep_sort_pos_1

Scan
14207

Method
FTMS; HCD

Score
67.99

m/z
656.34



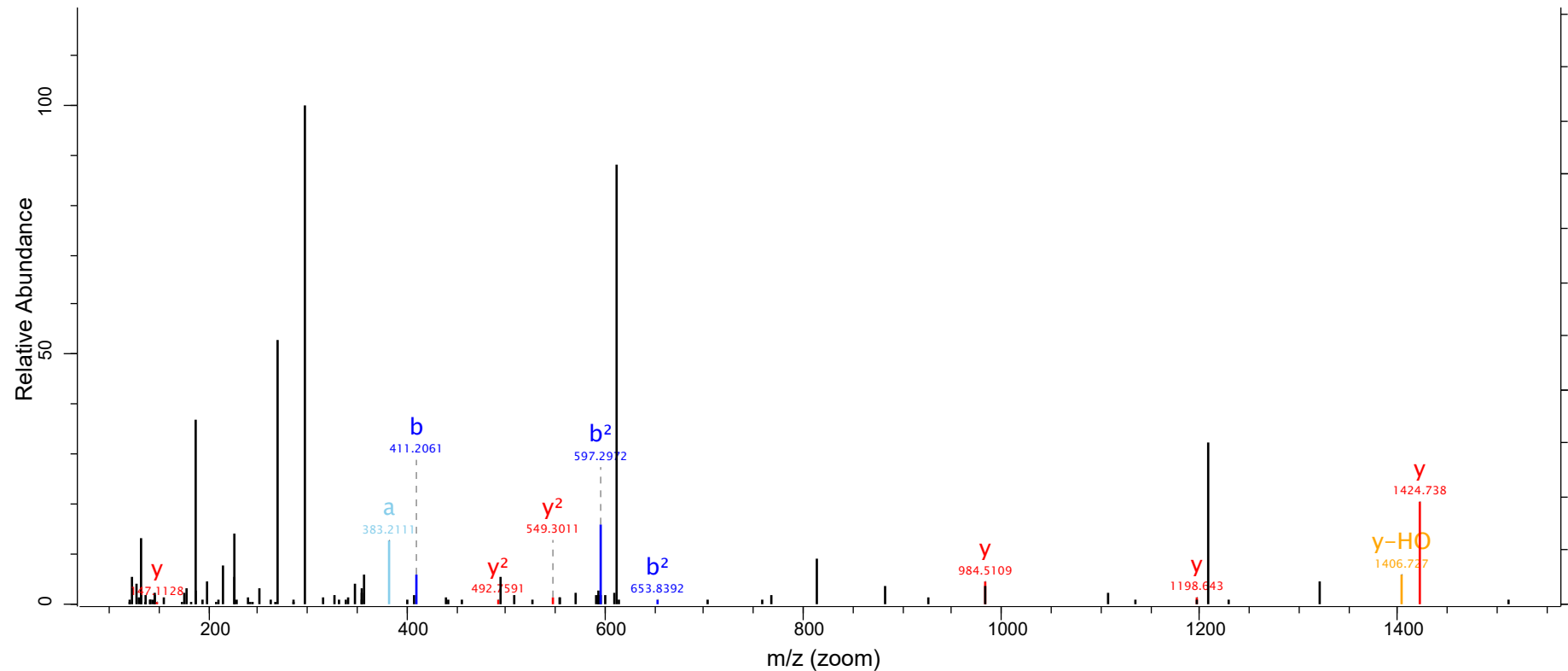
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agg_pep_sort_pos_3

Scan
18005

Method
FTMS; HCD

Score
49.93

m/z
612.65



bi - A L P E T L E N V L A S H S K -

Fragmentation diagram showing the sequence A L P E T L E N V L A S H S K - with b and y ions indicated by brackets below the sequence.

- b ions (blue brackets): b (L), b² (V), b² (L)
- y ions (red brackets): y (P), y (T), y² (L), y (E), y (S)

Raw File
agg_pep_sort_pos_3

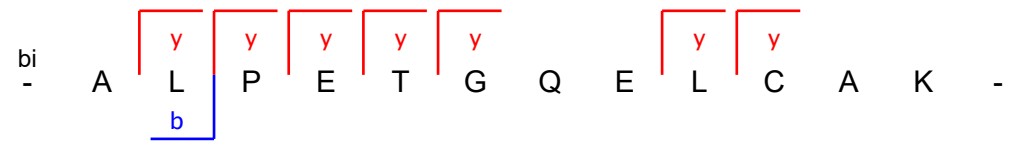
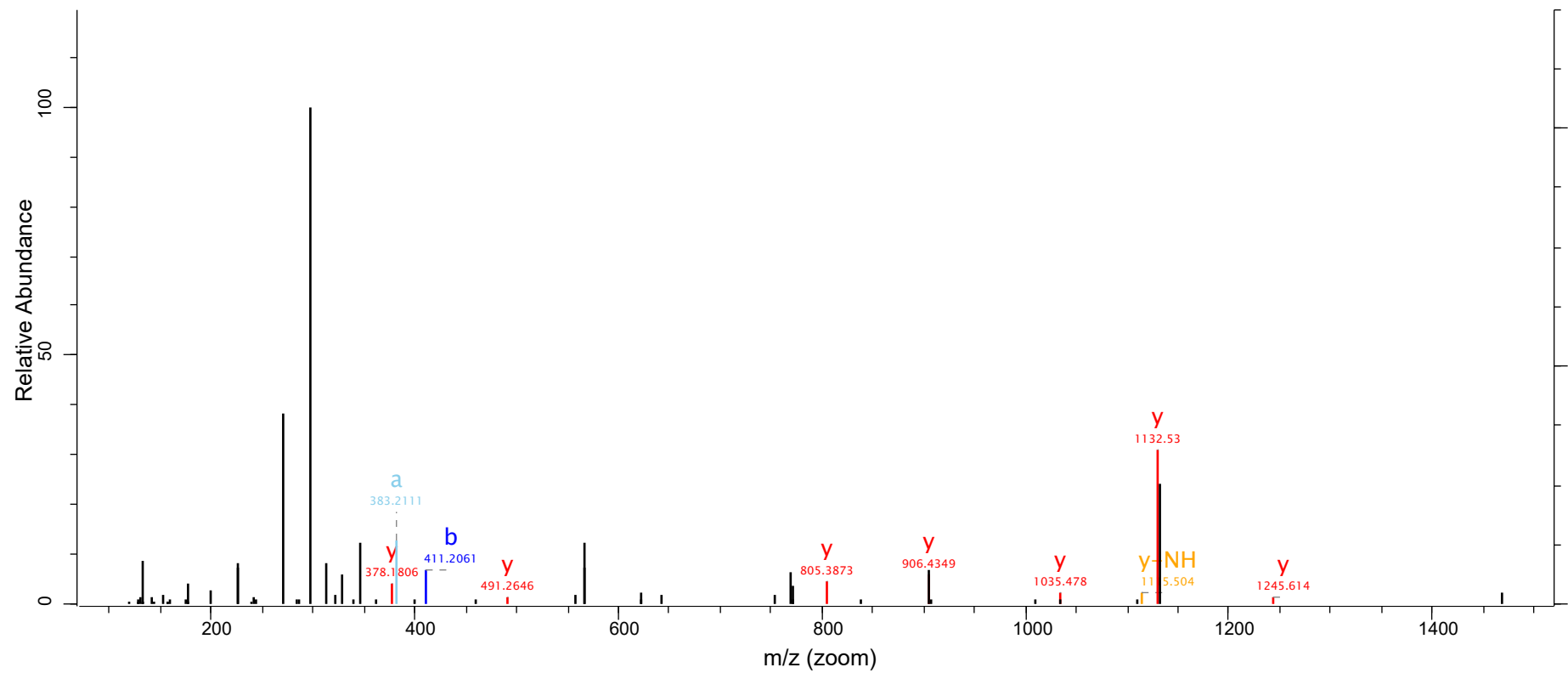
Scan
20012

Method
FTMS; HCD

Score
62.82

m/z
771.87

Gene names
CDK15



Raw File
agg_pep_sort_pos_1

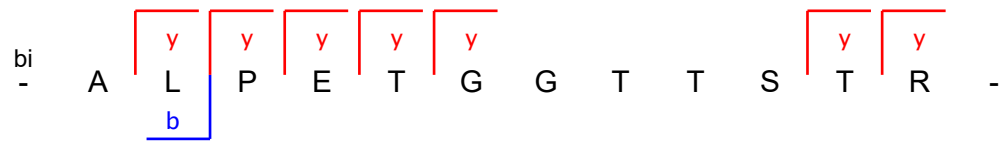
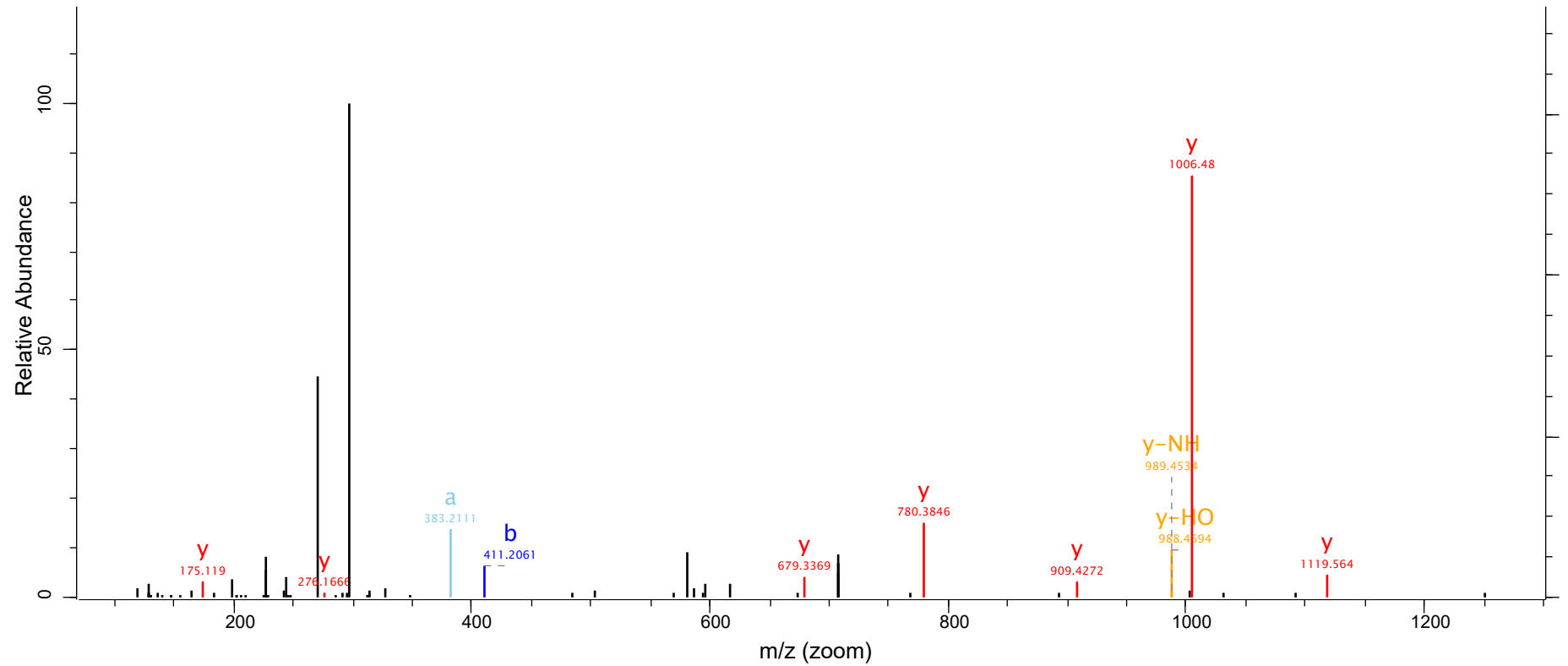
Scan
17383

Method
FTMS; HCD

Score
76.46

m/z
708.84

Gene names
CHCHD3



Raw File
agg_pep_sort_pos_1

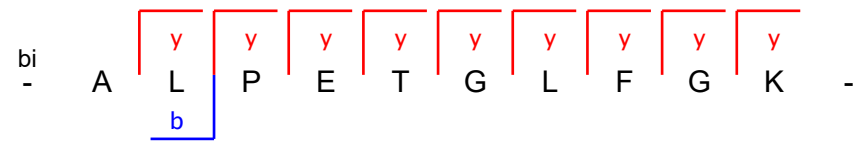
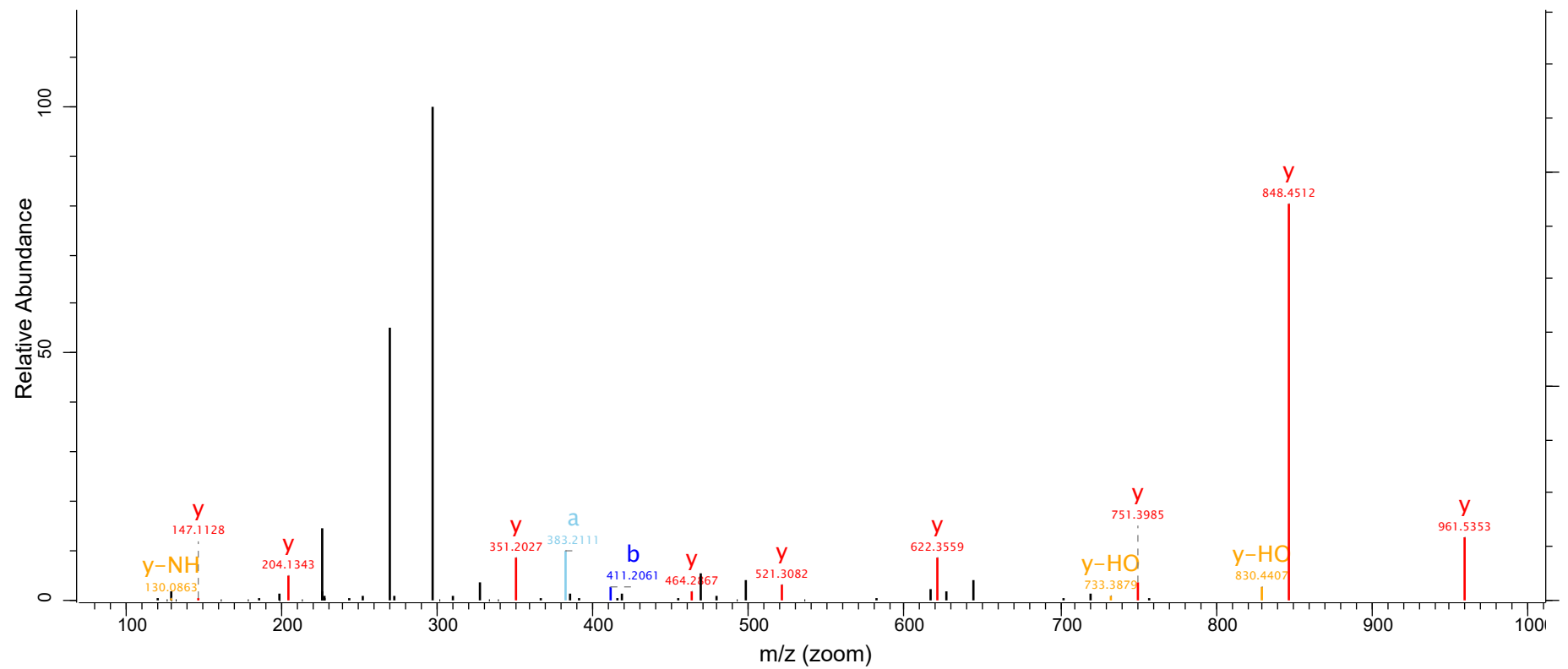
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Method
FTMS; HCD

Score
101.54

m/z
629.83

Gene names
CHMP3



Raw File
agg_pep_sort_pos_1

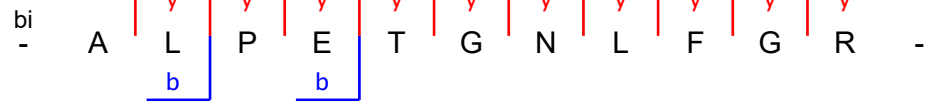
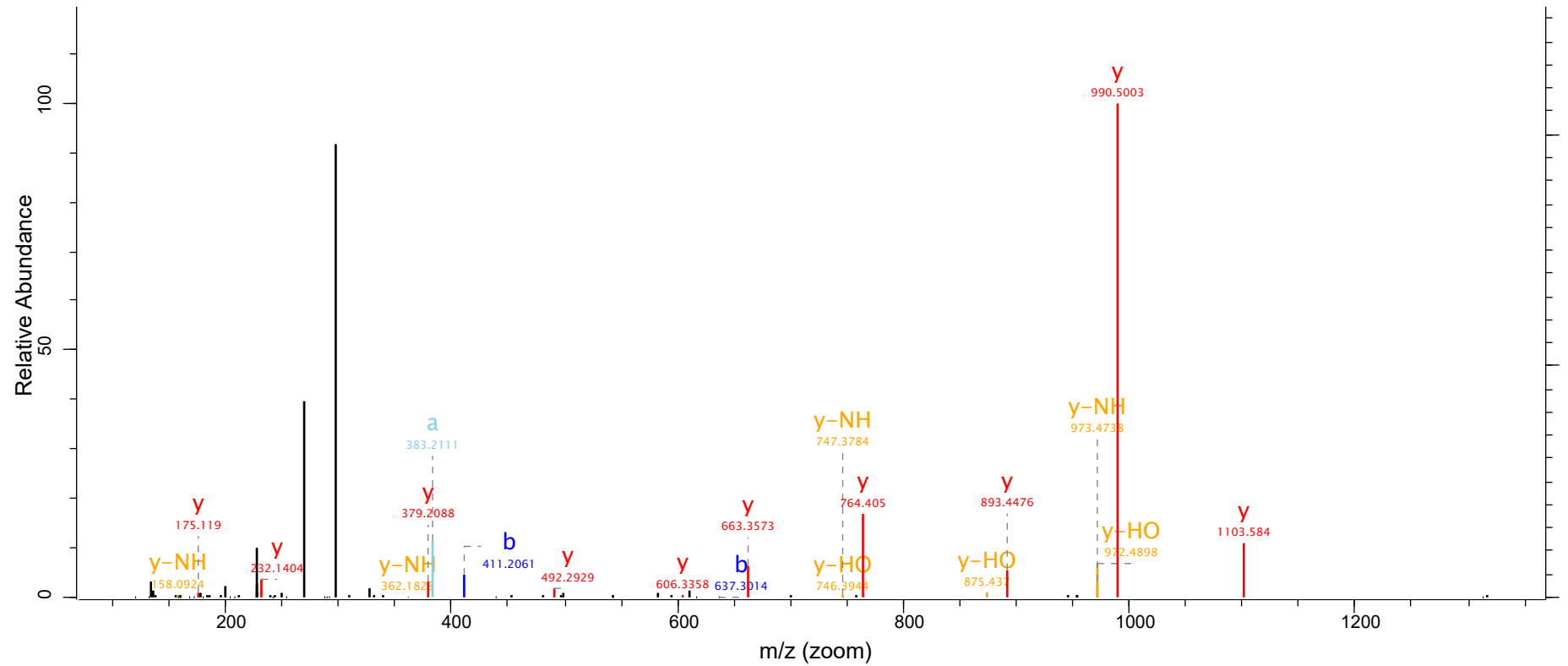
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26498

Method
FTMS; HCD

Score
130.75

m/z
700.85

Gene names
CHMP6



Raw File
agg_pep_sort_pos_2

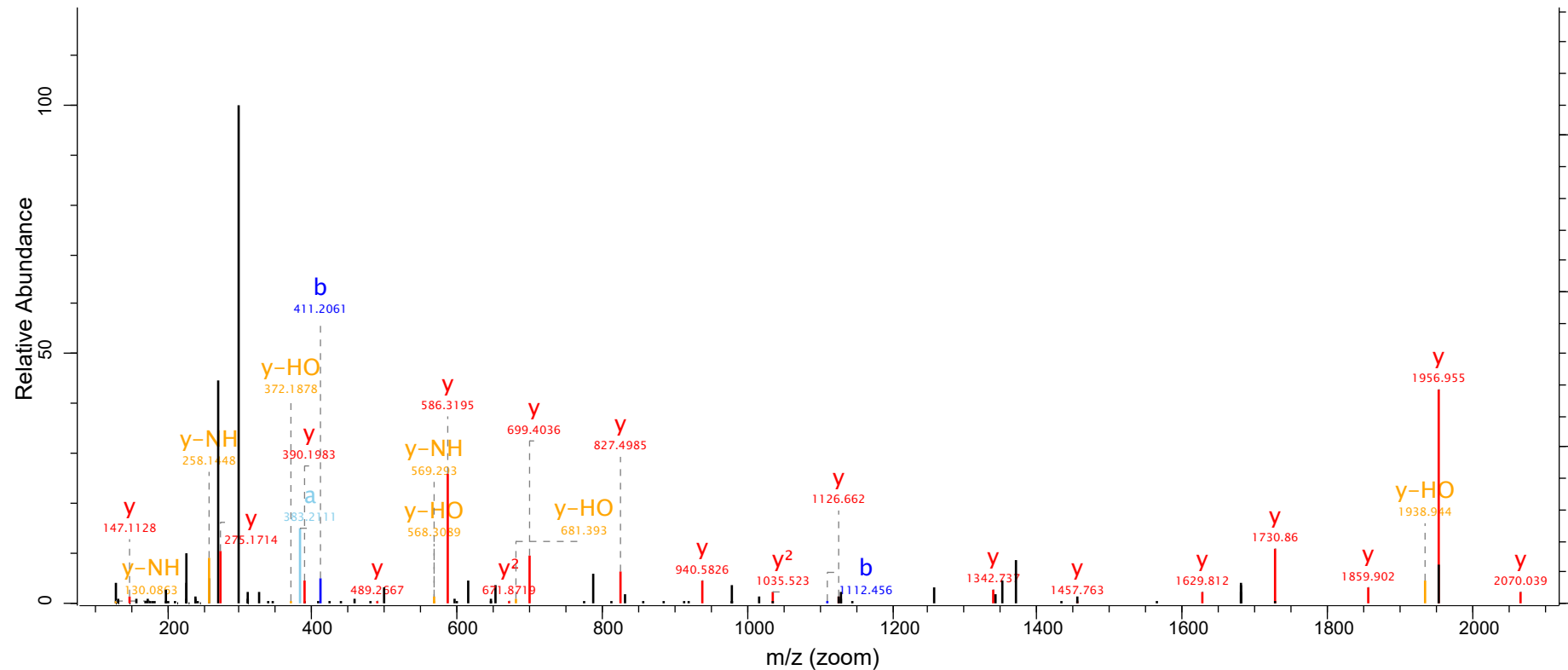
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Method
FTMS; HCD

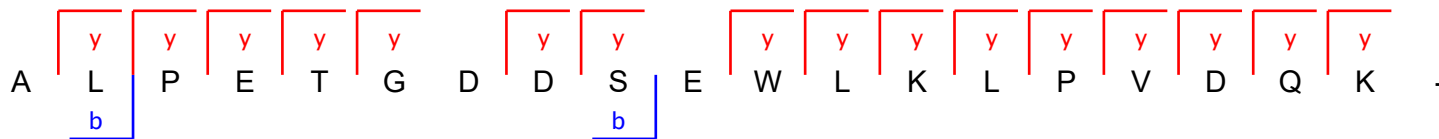
Score
117.65

m/z
790.06

Gene names
CKAP5



bi



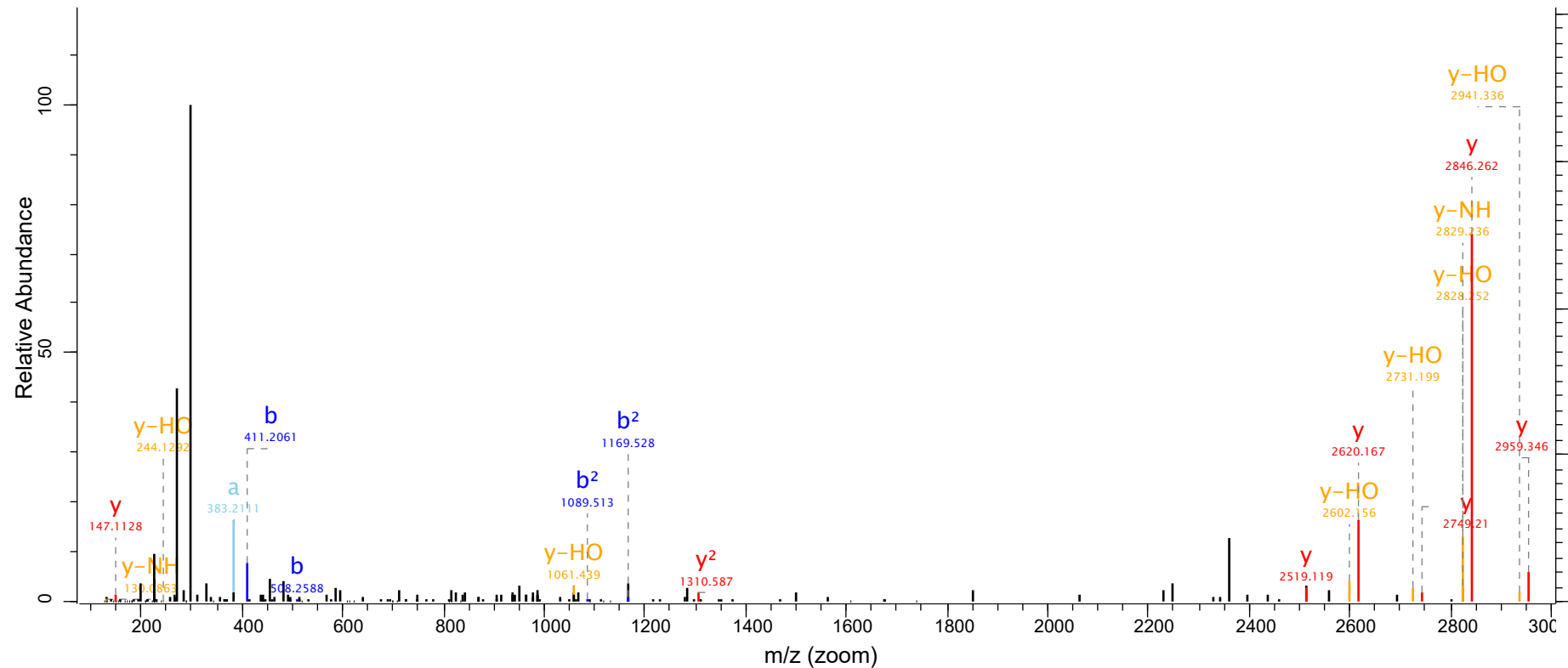
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agg_pep_sort_pos_2

Scan
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Method
FTMS; HCD

Score
75.65

m/z
815.12



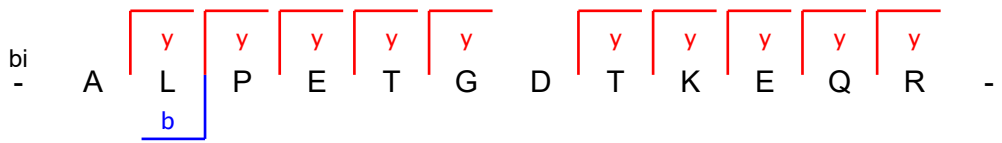
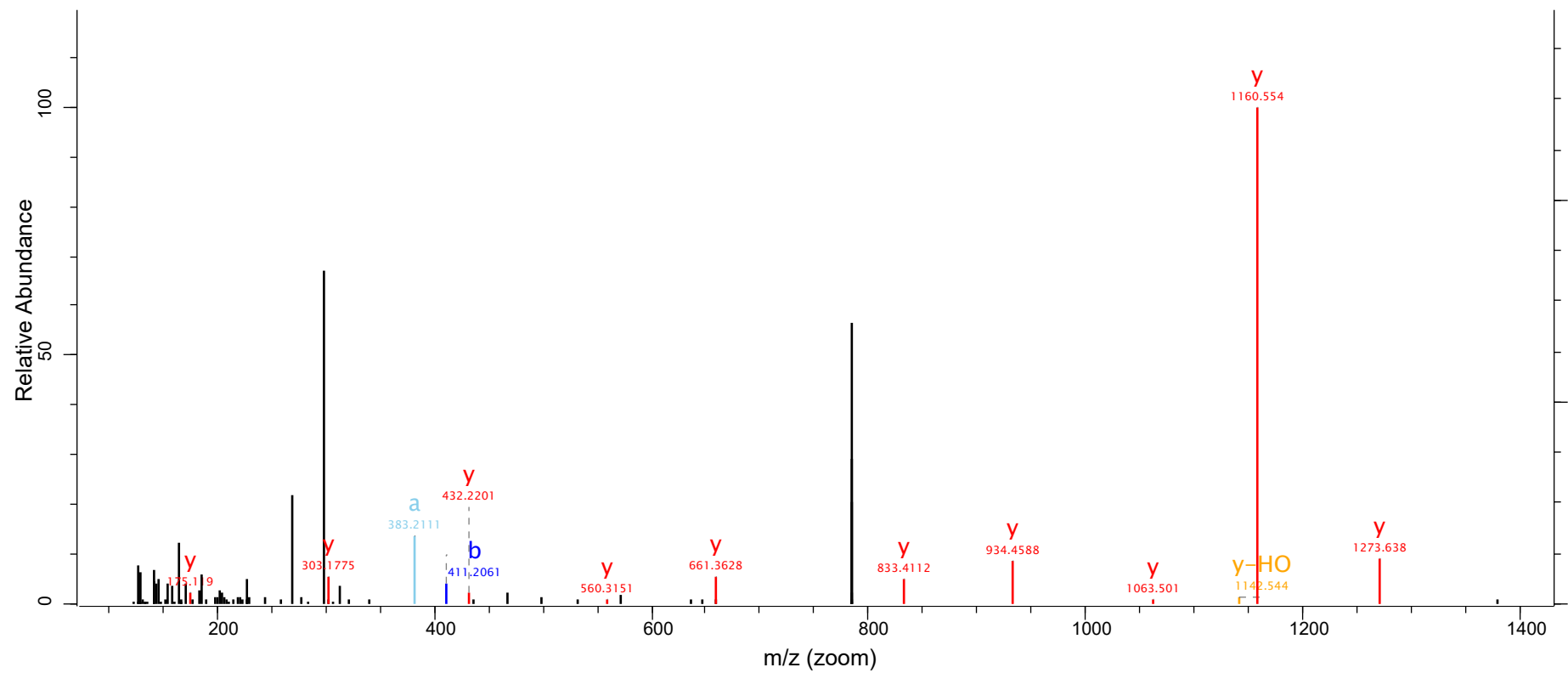
Raw File
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Scan
12993

Method
FTMS; HCD

Score
77.38

m/z
785.88



Raw File
agg_pep_sort_pos_1

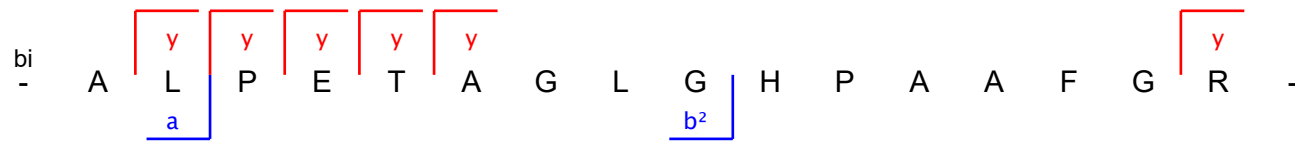
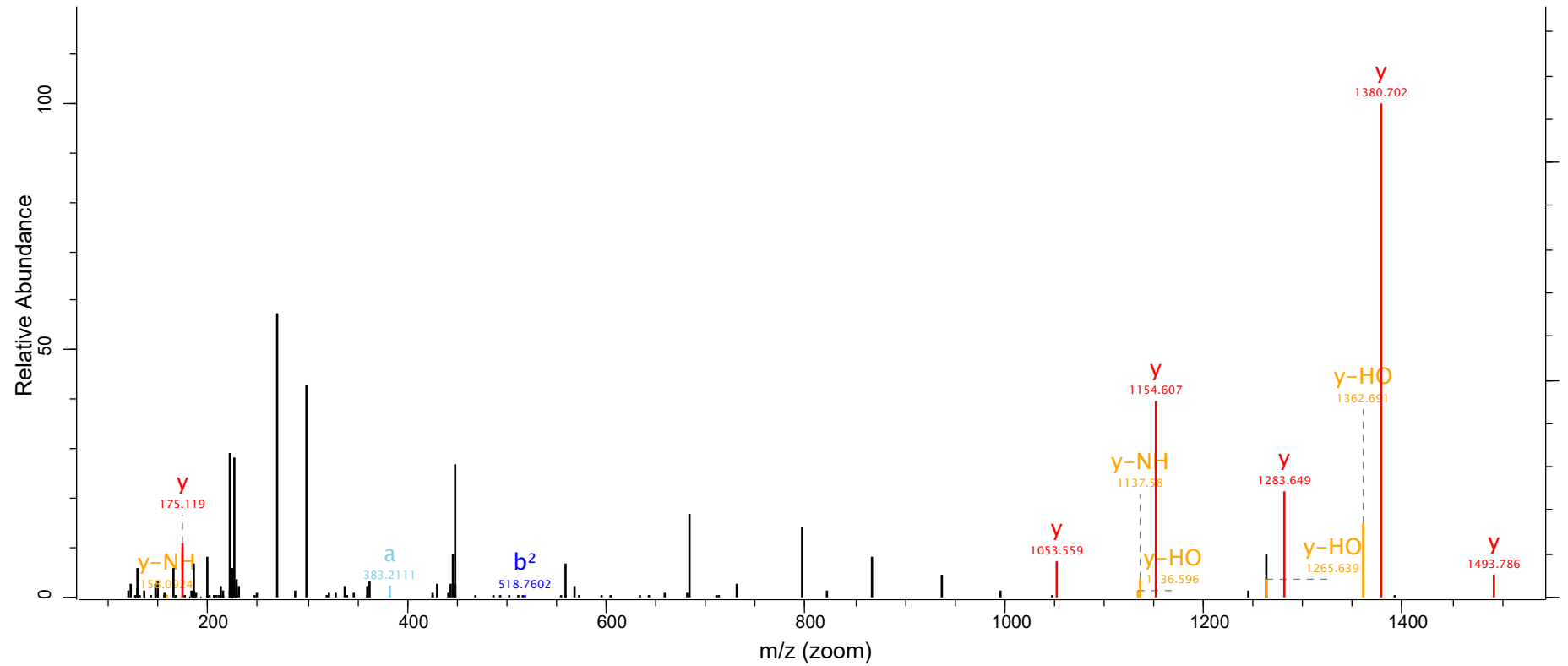
Scan
10383

Method
FTMS; HCD

Score
46.32

m/z
448.48

Gene names
DDAH1



Raw File
agg_pep_sort_pos_1

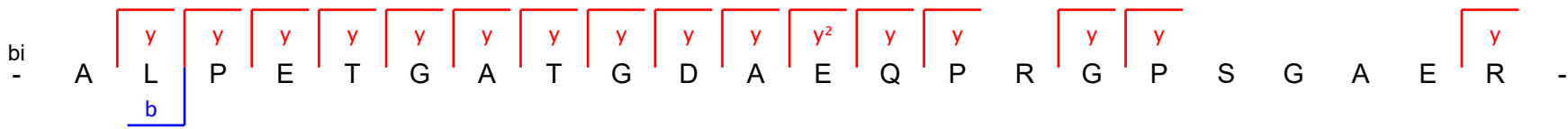
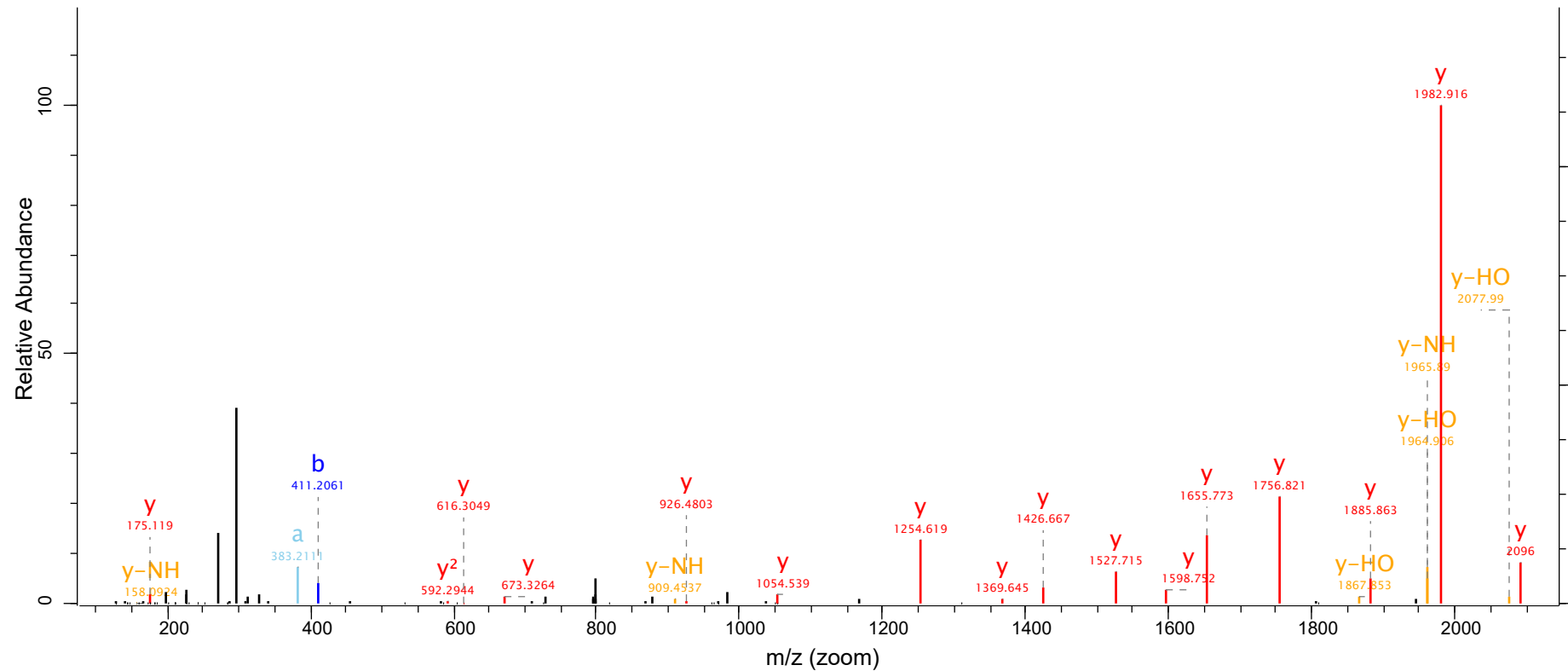
Scan
15482

Method
FTMS; HCD

Score
115.46

m/z
798.71

Gene names
DNMT1P1



Raw File
agg_pep_sort_pos_1

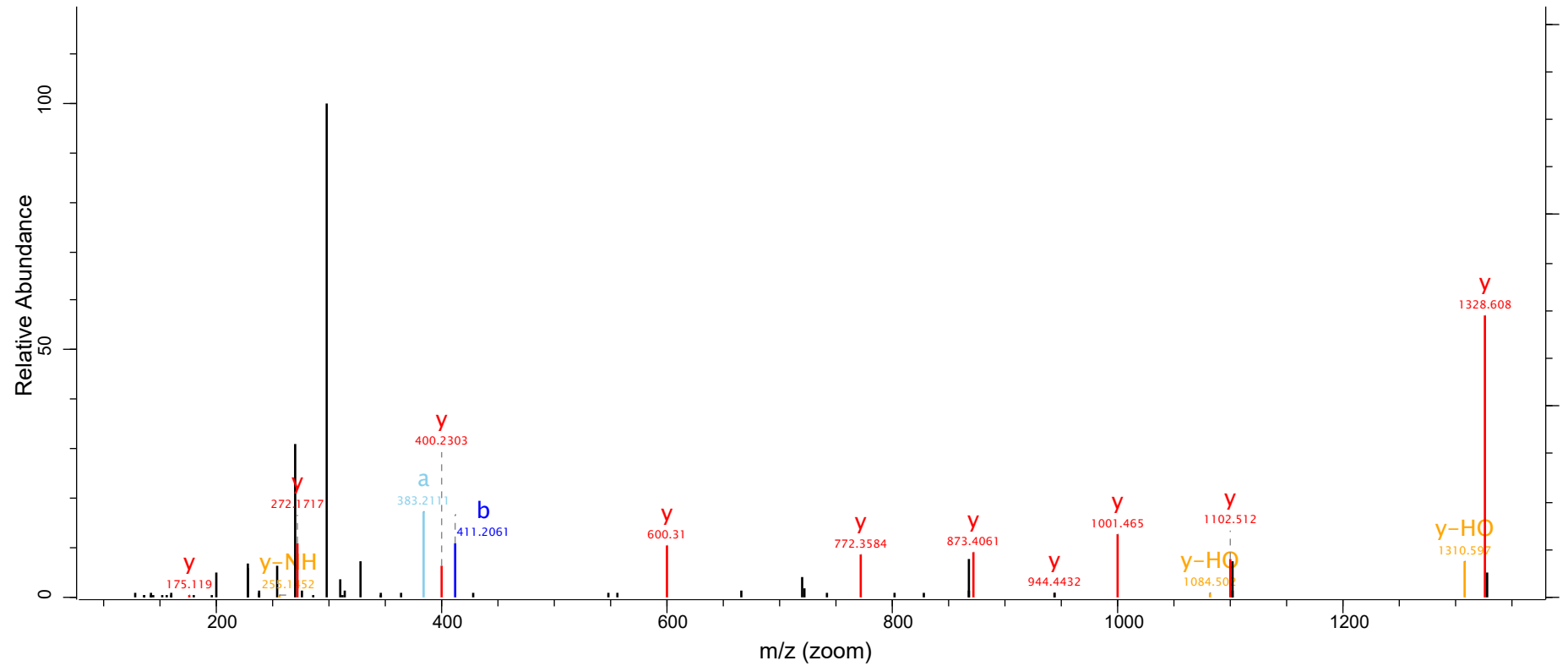
Scan
18883

Method
FTMS; HCD

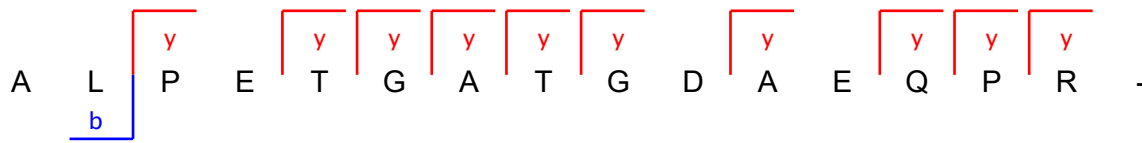
Score
82.17

m/z
870.41

Gene names
DNTTIP1



bi



Raw File
agg_pep_sort_pos_2

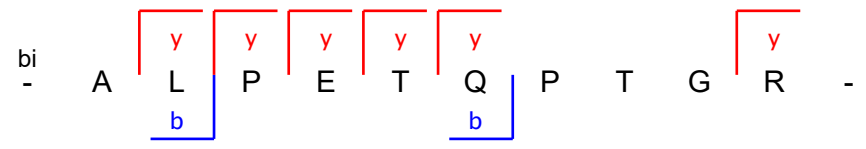
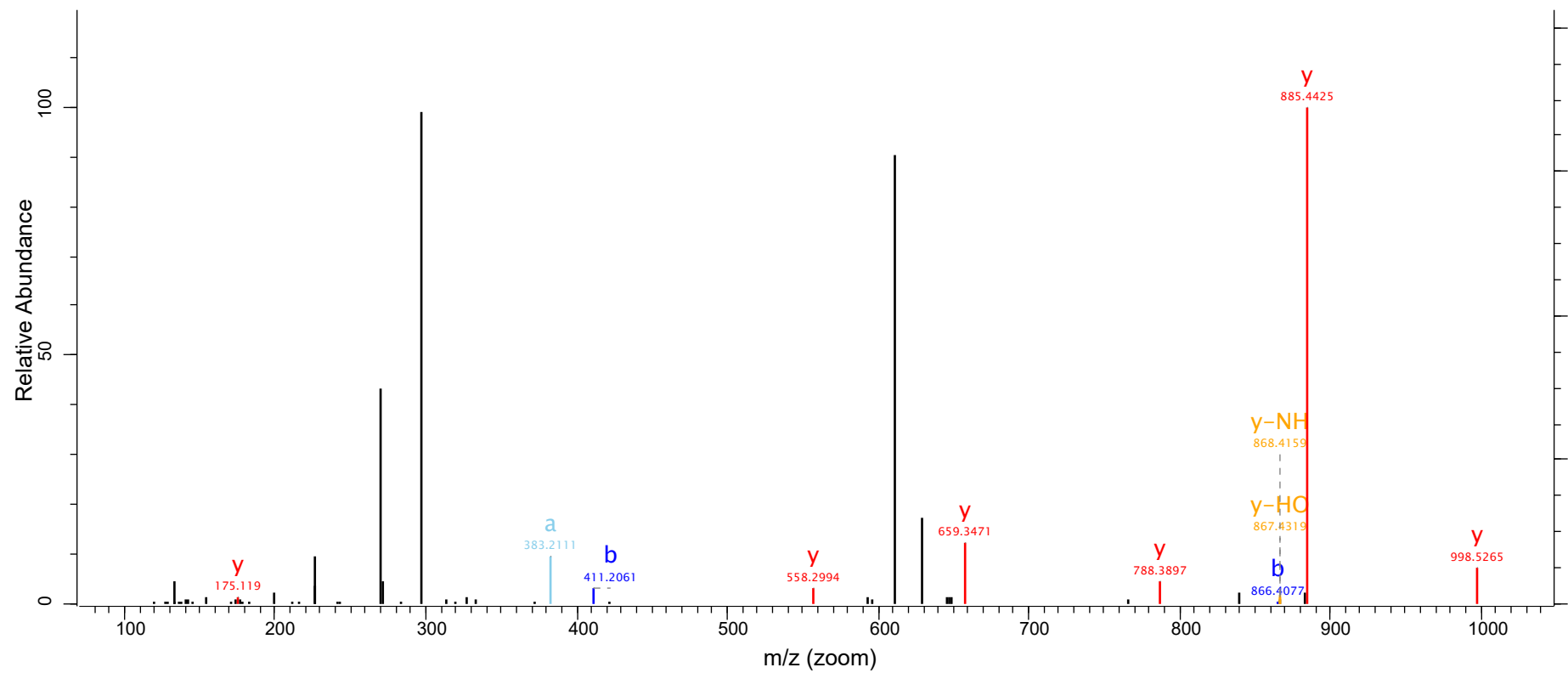
Scan
17592

Method
FTMS; HCD

Score
67.9

m/z
648.32

Gene names
DPEP3



Raw File
agg_pep_sort_pos_2

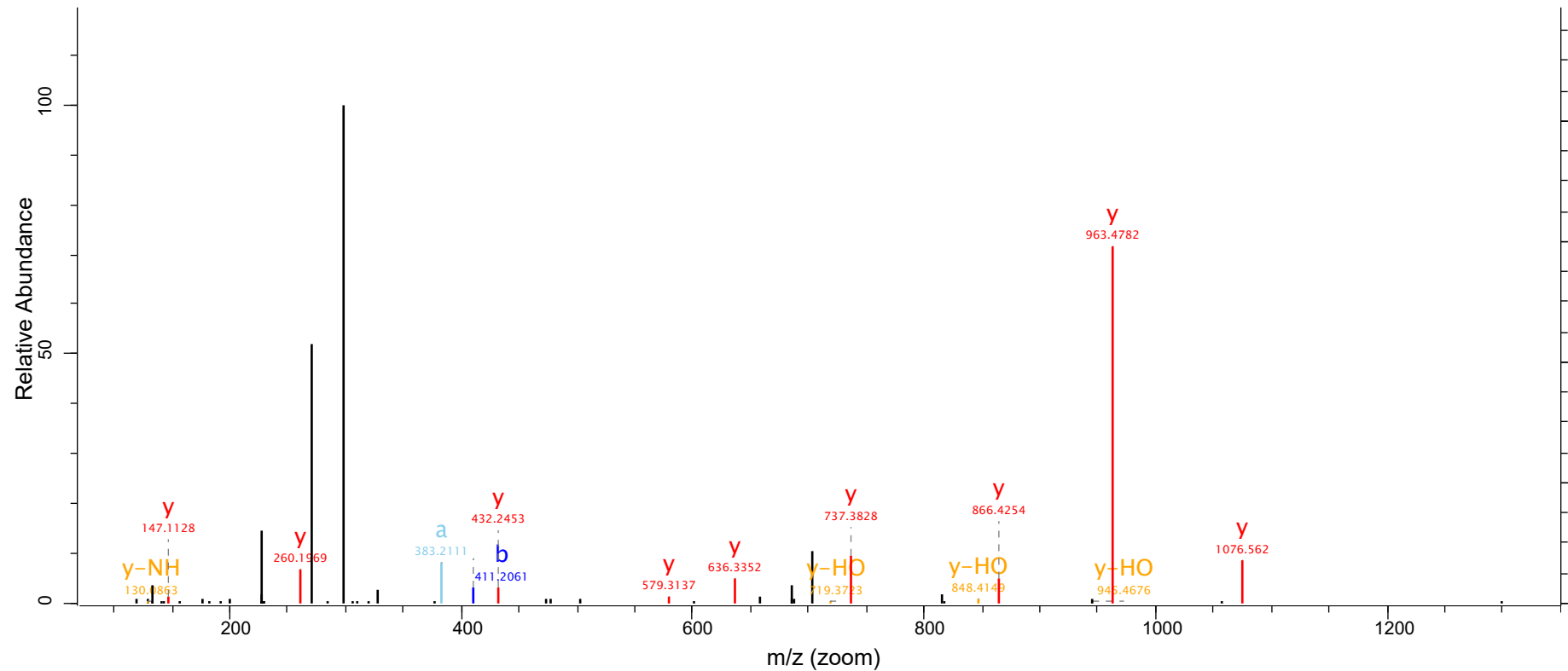
Scan
25042

Method
FTMS; HCD

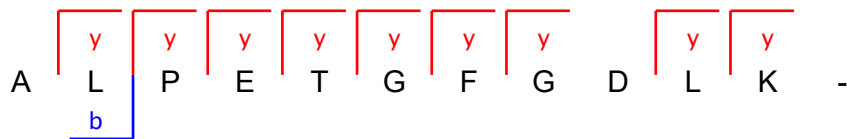
Score
94.69

m/z
687.84

Gene names
EEF1B2



bi



Raw File
agg_pep_sort_pos_1

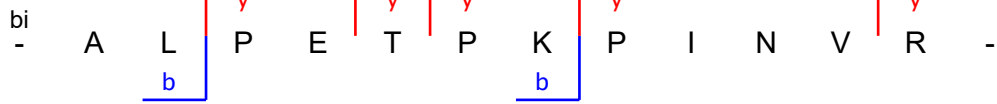
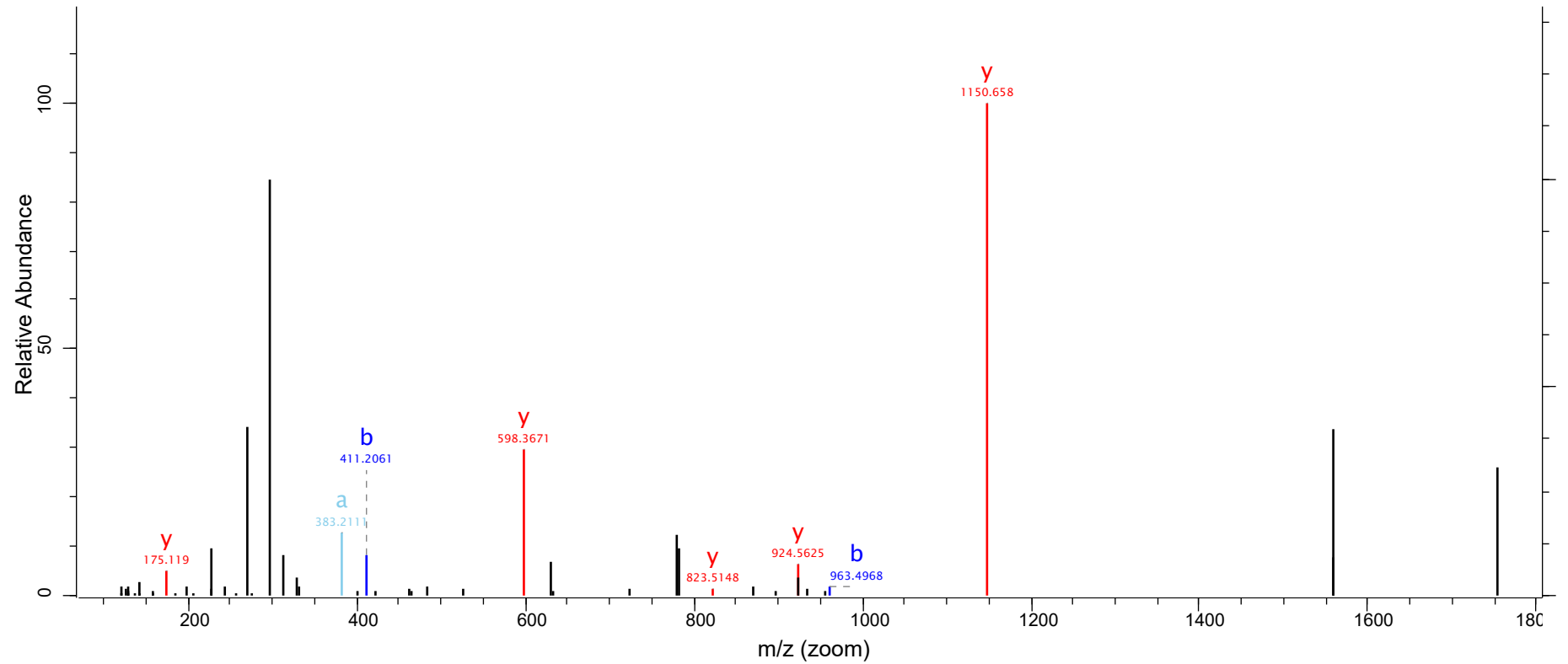
Scan
17488

Method
FTMS; HCD

Score
55.55

m/z
781.43

Gene names
EZR;RDX



Raw File
agg_pep_sort_pos_3

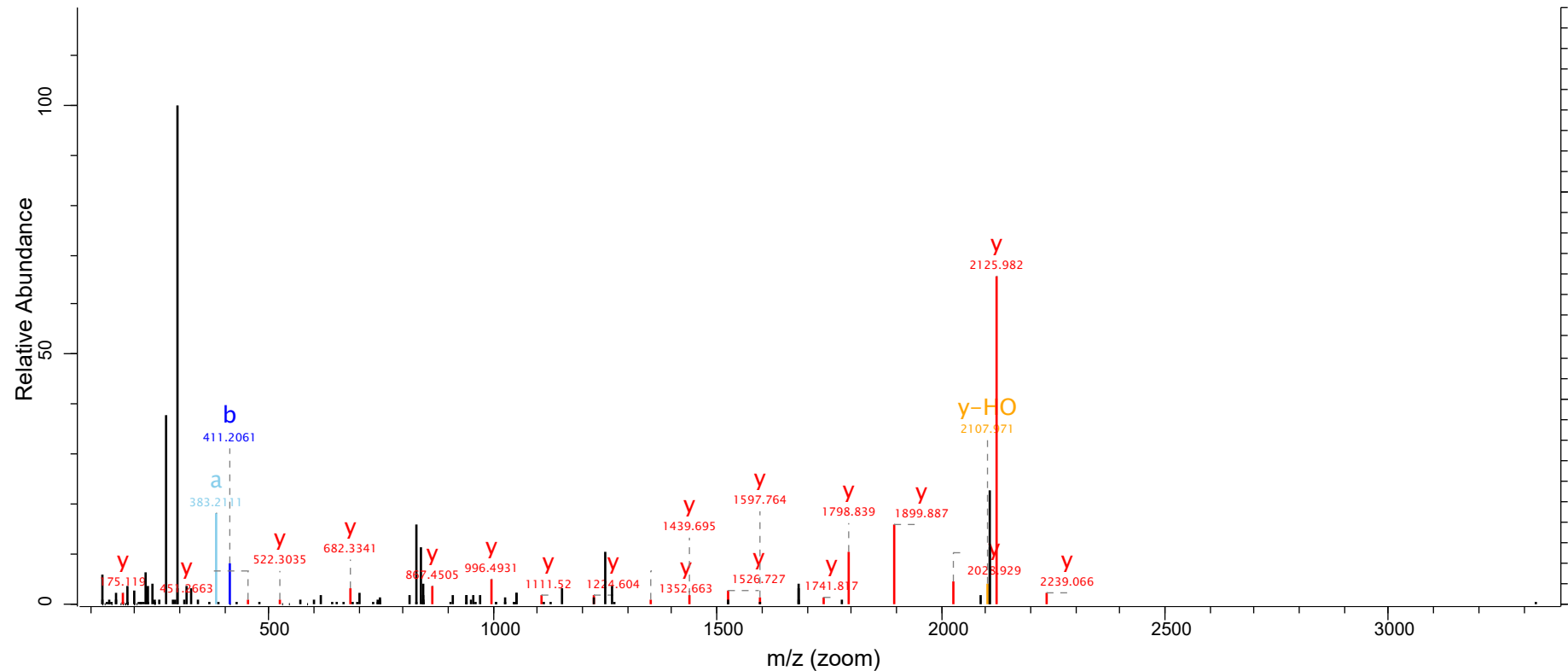
Scan
18442

Method
FTMS; HCD

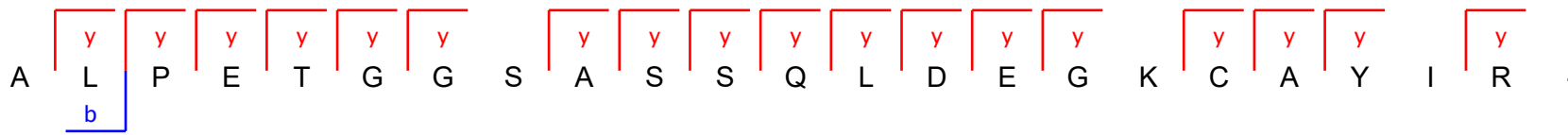
Score
77.68

m/z
846.4

Gene names
FAM129A



bi



Raw File
agg_pep_sort_pos_1

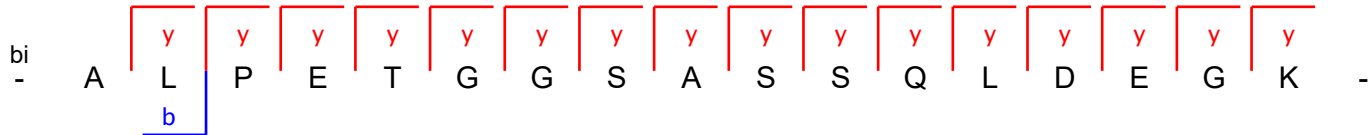
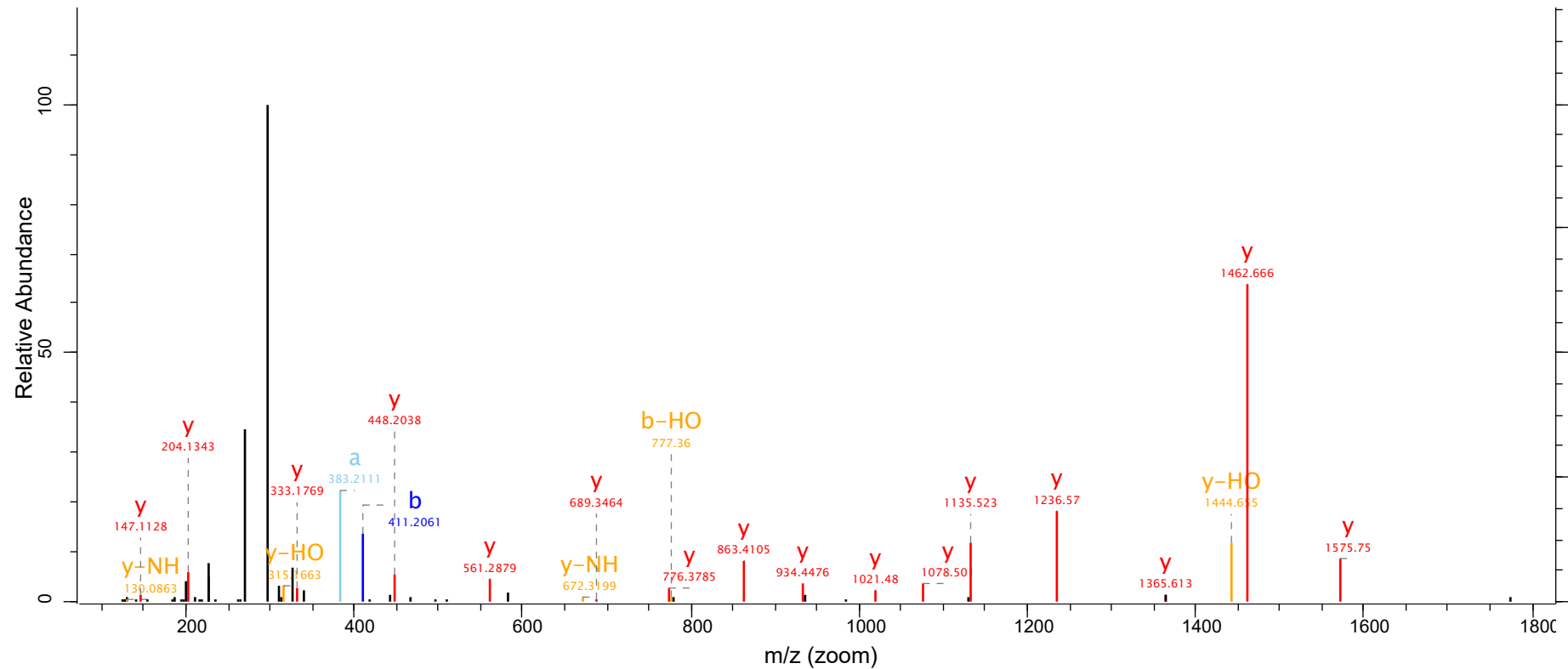
Scan
19601

Method
FTMS; HCD

Score
143.86

m/z
936.94

Gene names
FAM129A



Raw File
agg_pep_sort_pos_3

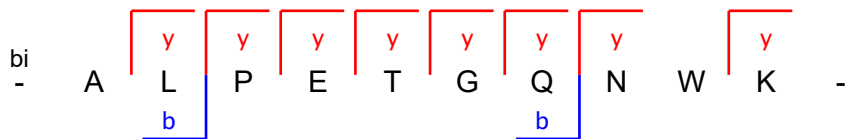
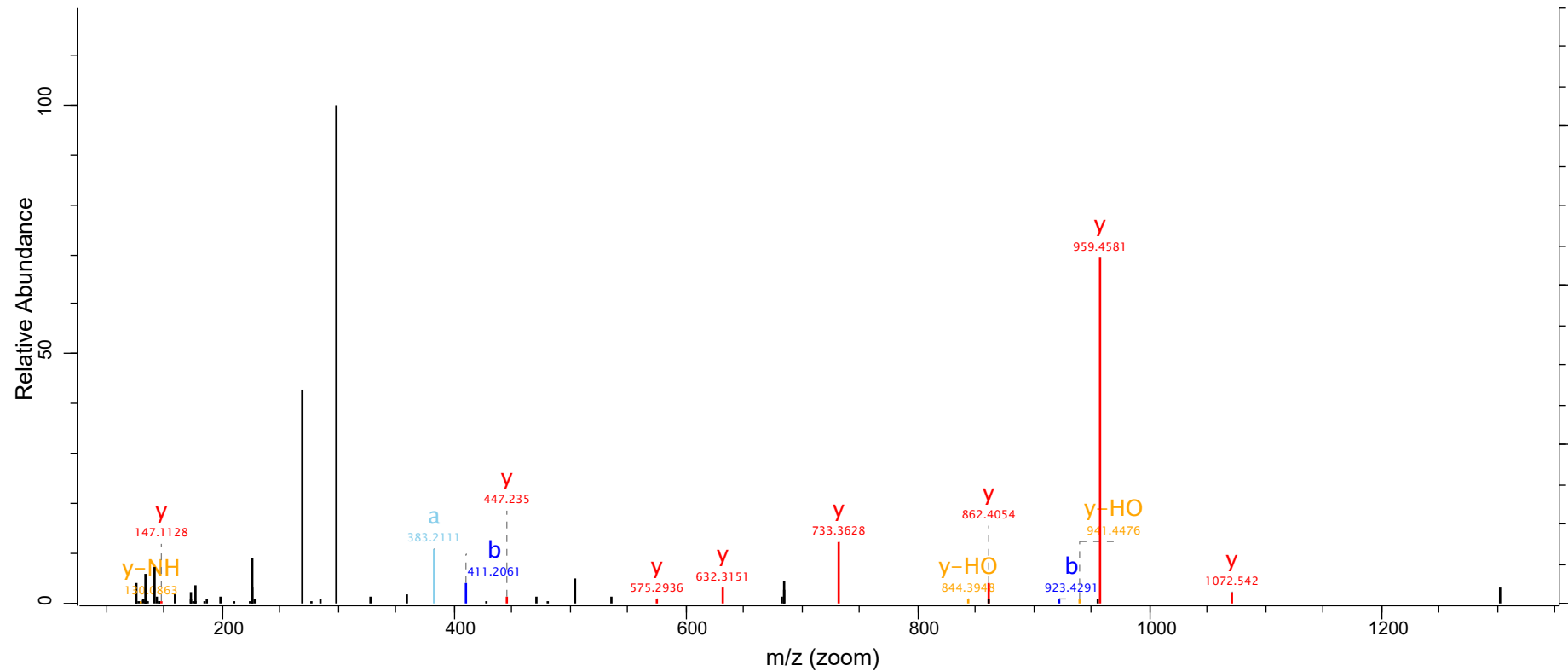
Scan
21095

Method
FTMS; HCD

Score
80.24

m/z
685.33

Gene names
FAM216B



Raw File
agg_pep_sort_pos_2

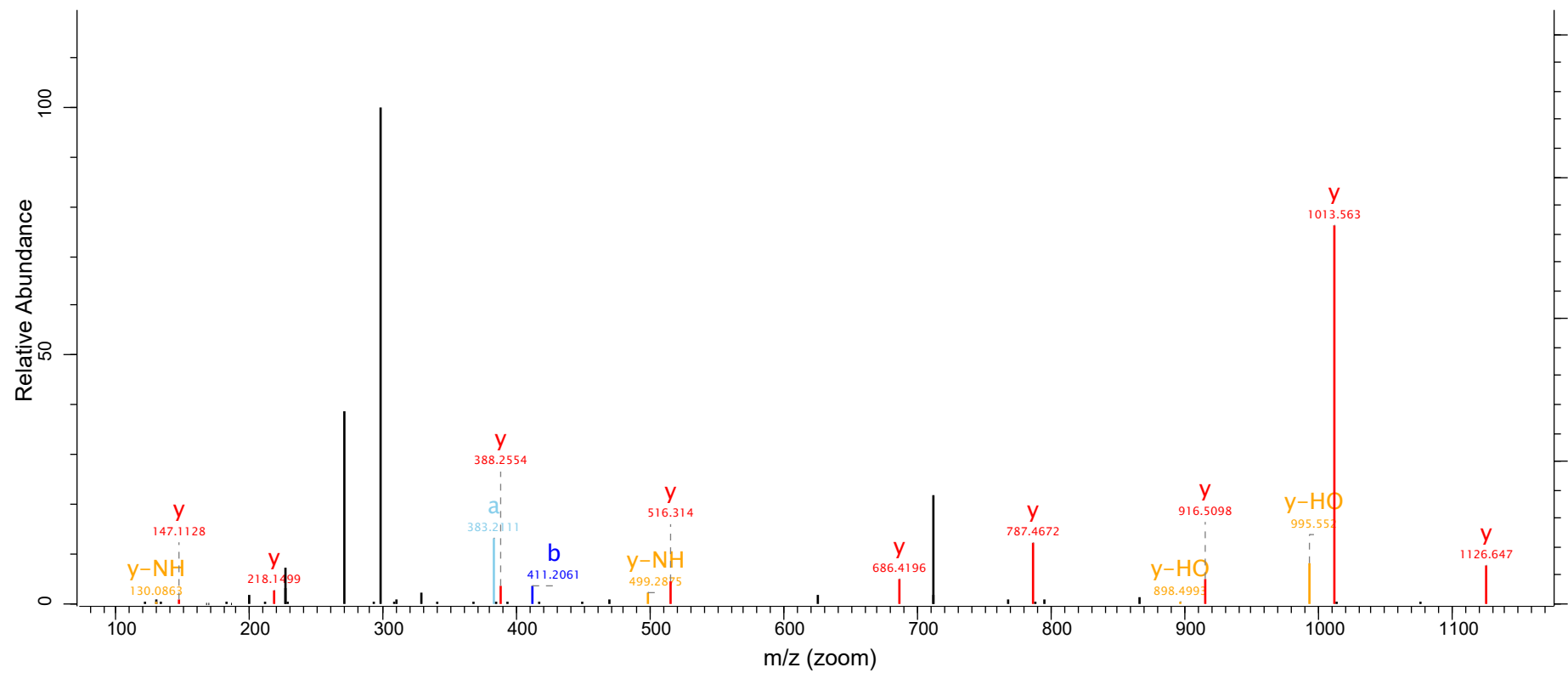
Scan
23260

Method
FTMS; HCD

Score
122.13

m/z
712.88

Gene names
FEN1



Raw File
agg_pep_sort_pos_1

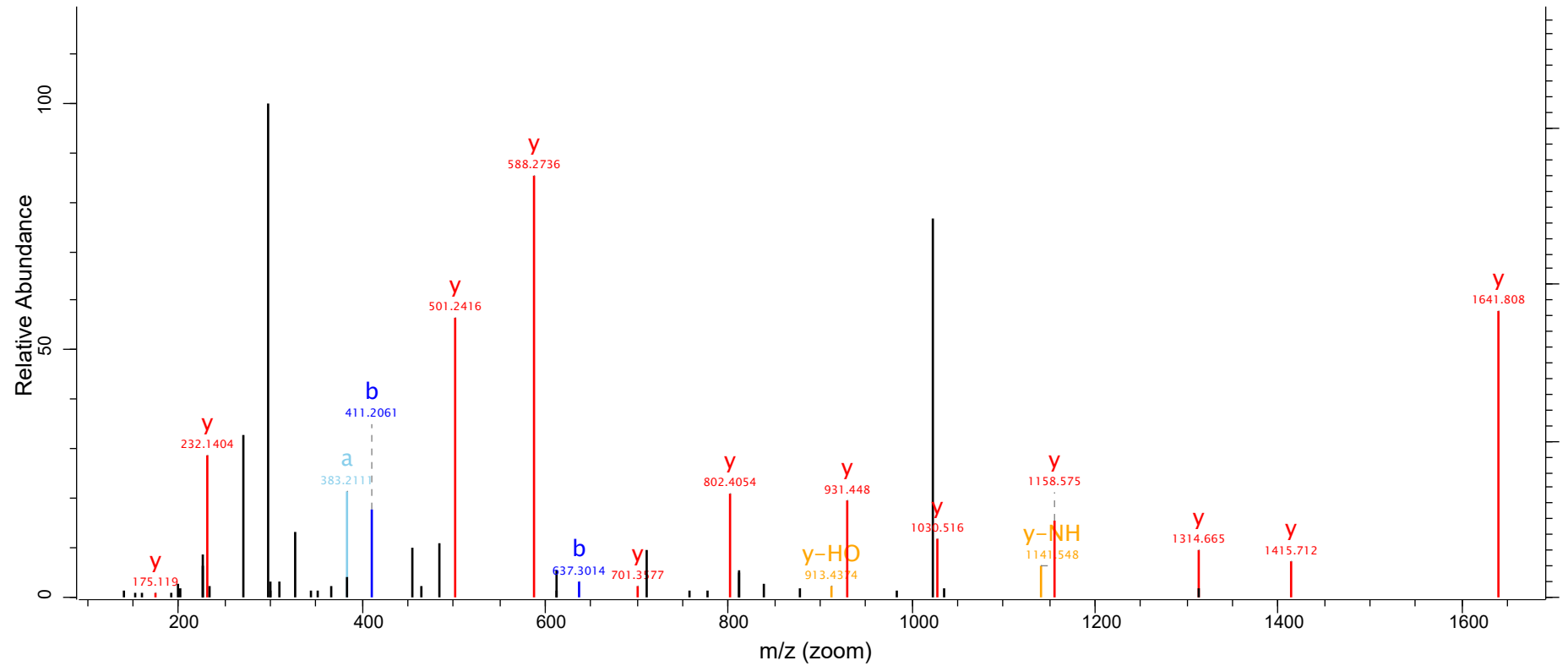
Scan
24289

Method
FTMS; HCD

Score
124.86

m/z
1027.01

Gene names
FKBP1A



bi



Raw File
agg_pep_sort_pos_2

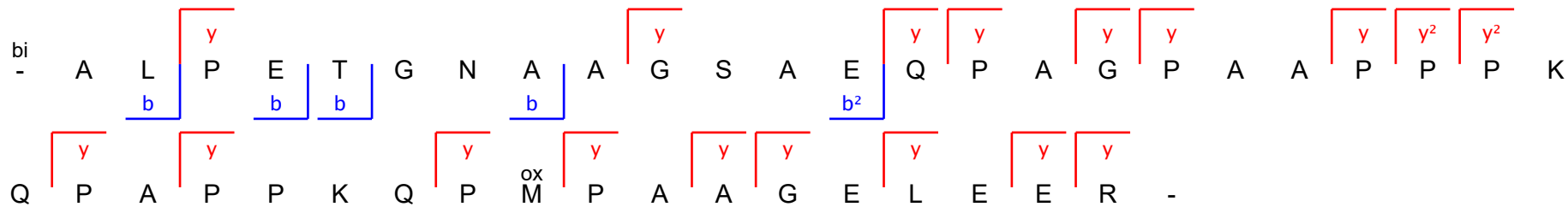
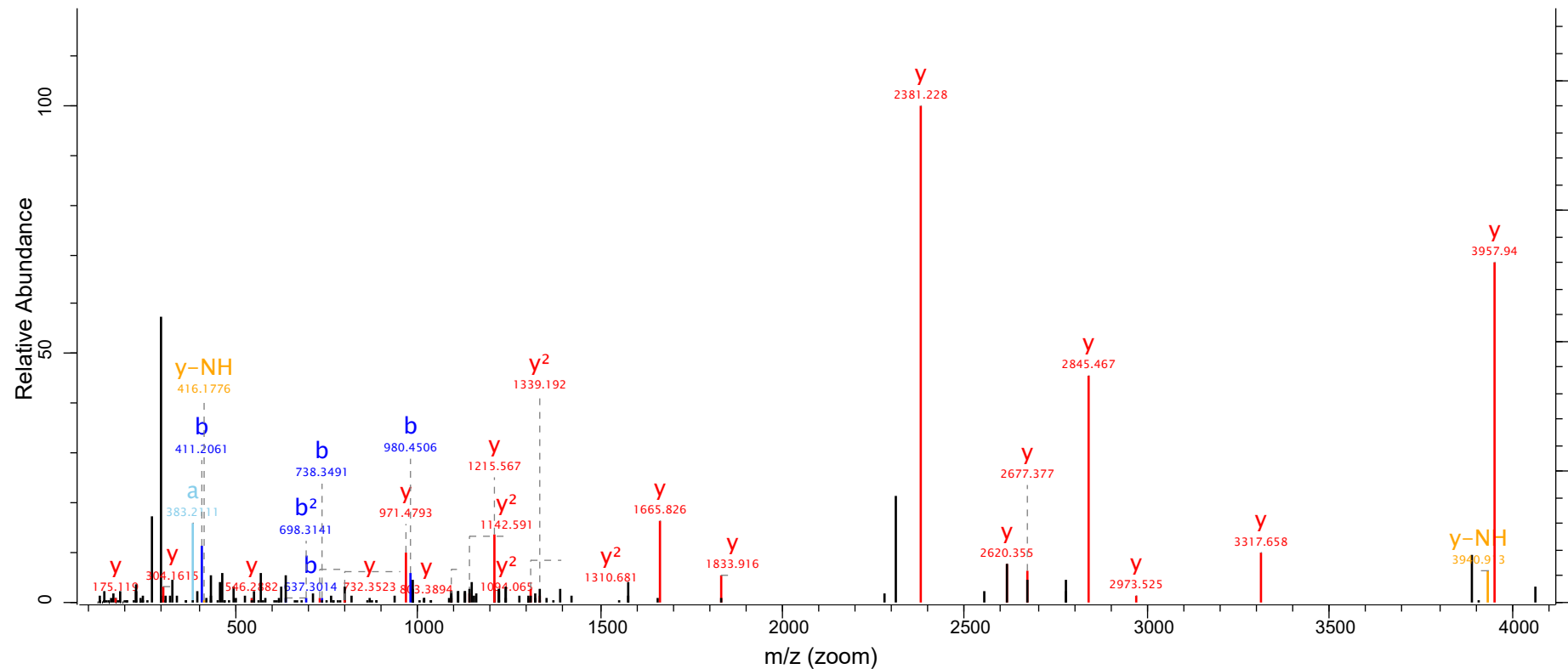
Scan
15998

Method
FTMS; HCD

Score
70.93

m/z
1093.29

Gene names
FMNL1



Raw File
agg_pep_sort_pos_2

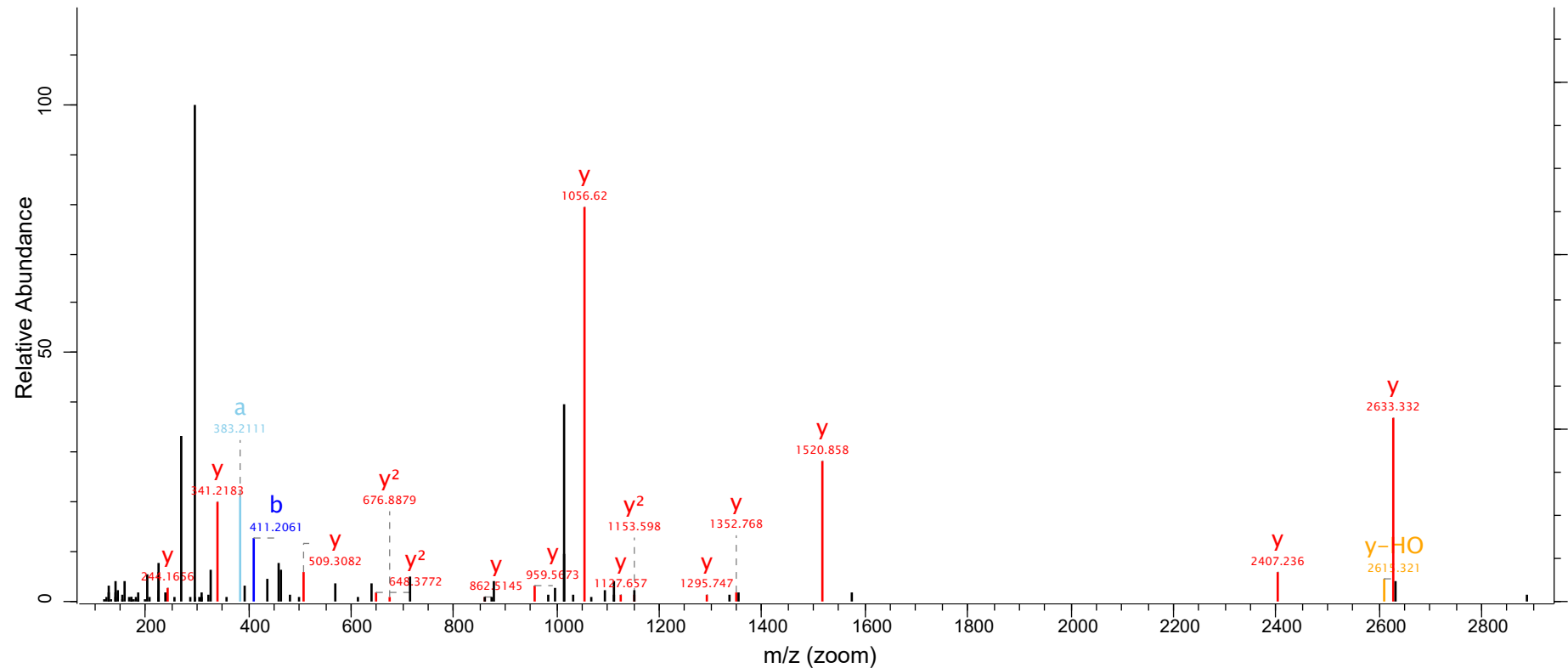
Scan
16015

Method
FTMS; HCD

Score
41.56

m/z
1015.52

Gene names
FMNL1



Raw File
agg_pep_sort_pos_3

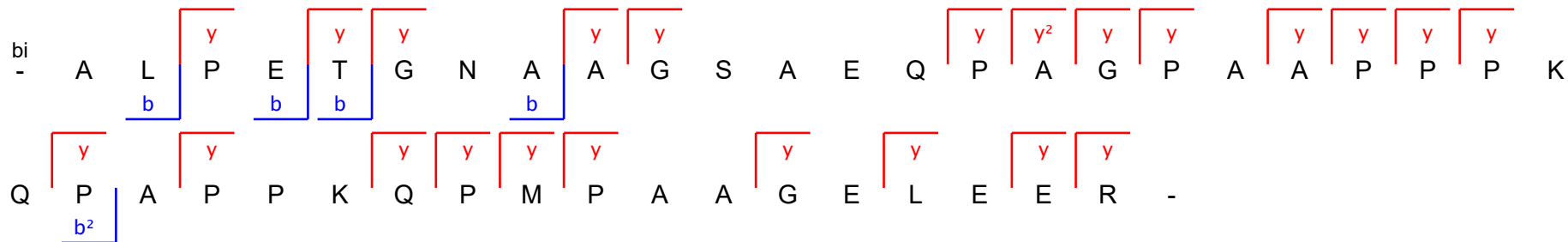
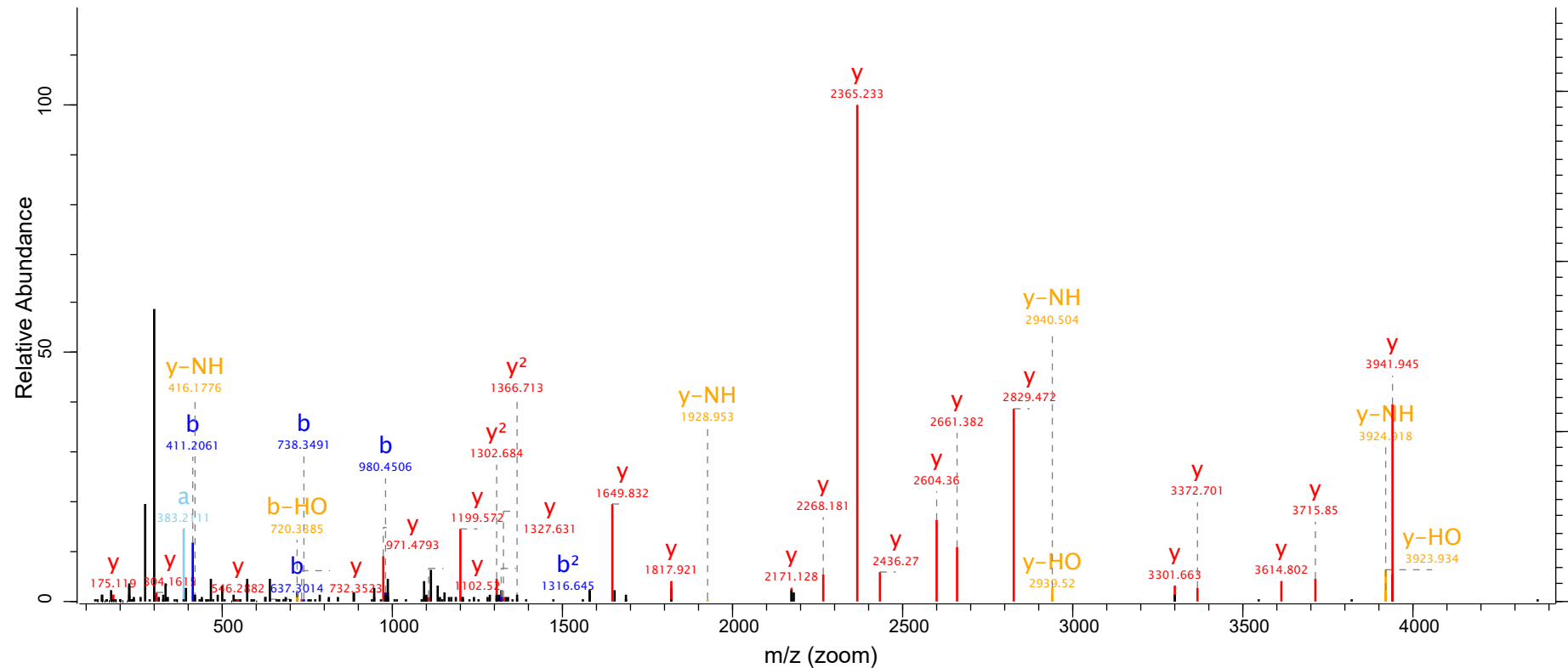
Scan
17255

Method
FTMS; HCD

Score
82.31

m/z
1089.54

Gene names
FMNL1



Raw File
agg_pep_sort_pos_3

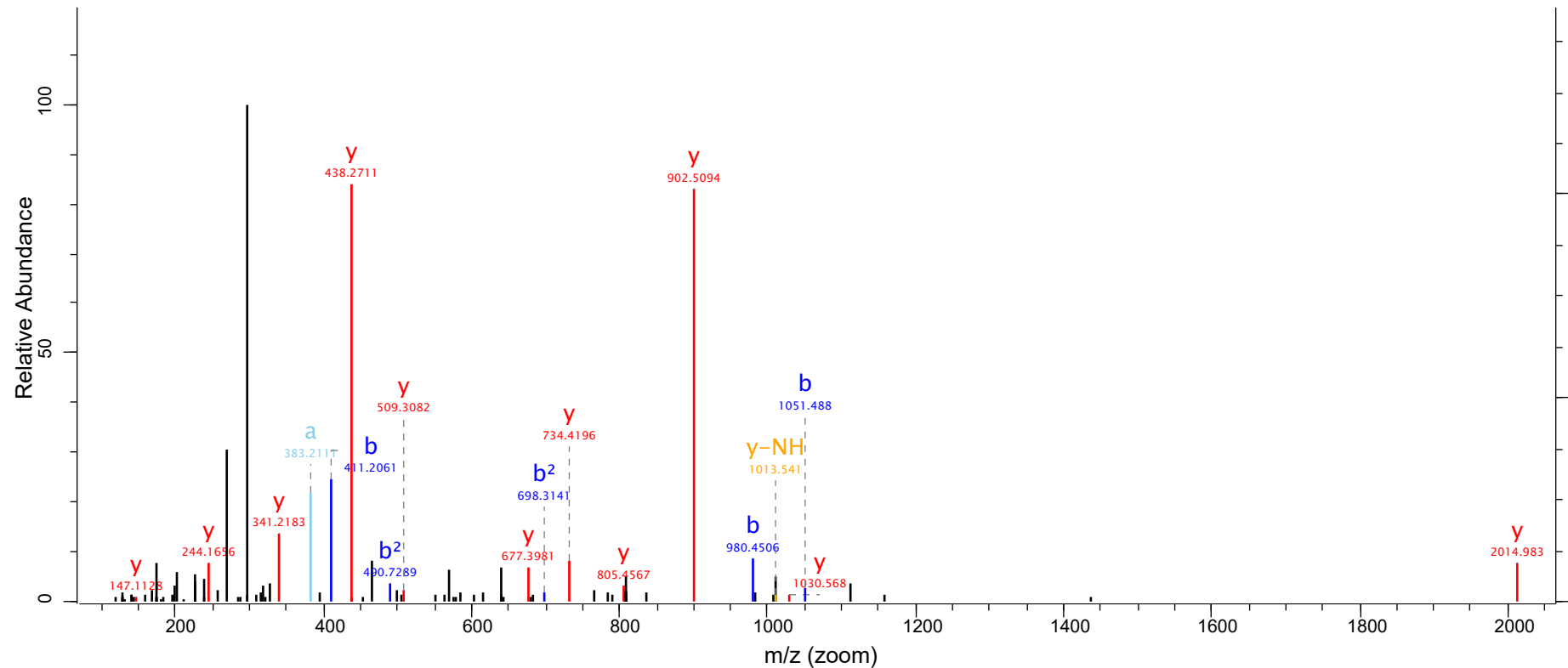
Scan
18903

Method
FTMS; HCD

Score
64.52

m/z
809.4

Gene names
FMNL1



bi

A L P E T G N A A G S A E Q P A G P A A P P P K

b

b

b

b²

-

Raw File
agg_pep_sort_pos_2

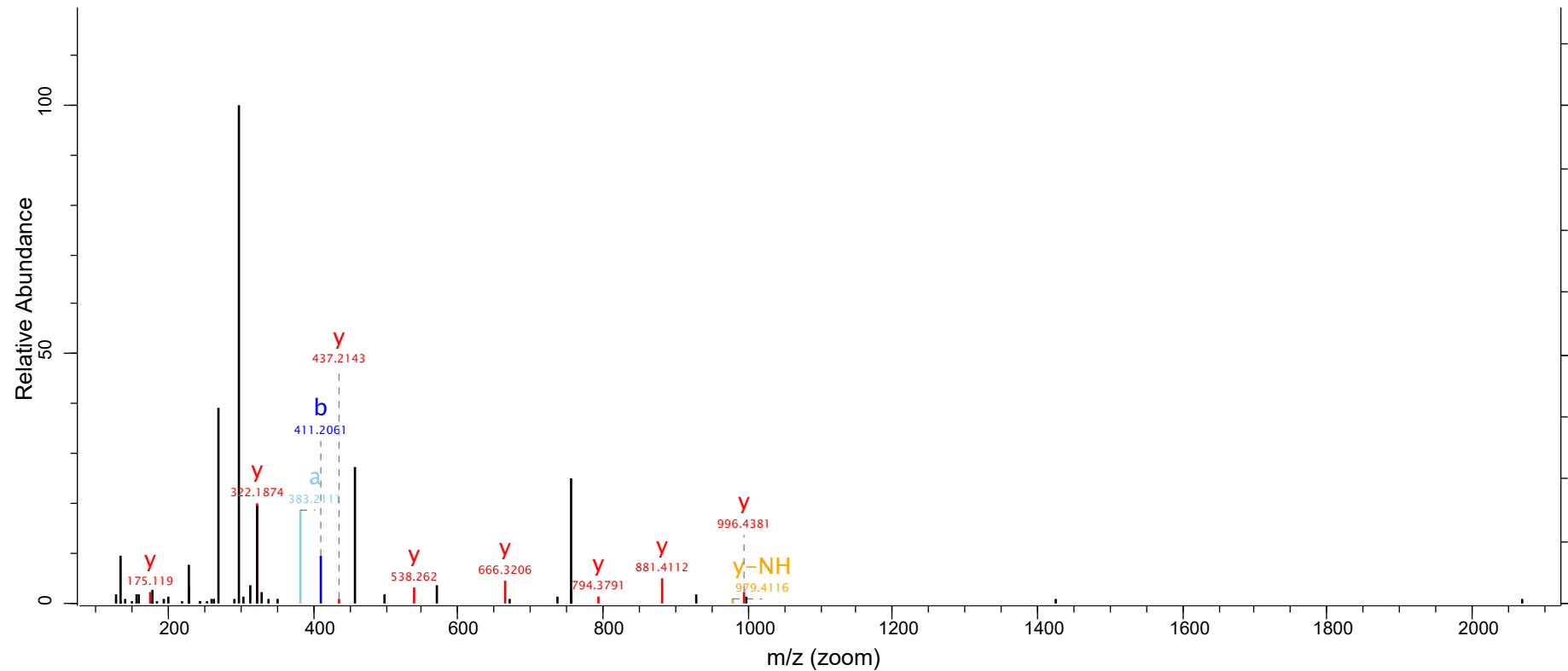
Scan
19983

Method
FTMS; HCD

Score
40.35

m/z
756.66

Gene names
FMNL2



bi
- A L P E T G N A G S ox M D S Q Q T D F R -

Fragmentation diagram showing b-ions (blue) and y-ions (red) for the peptide sequence: A L P E T G N A G S ox M D S Q Q T D F R -.

The b-ion ladder is shown for the first three residues: A (m/z 133), L (m/z 175), and P (m/z 217). The y-ion ladder is shown for the last eight residues: M (m/z 147), D (m/z 189), S (m/z 231), Q (m/z 273), Q (m/z 315), T (m/z 357), D (m/z 399), and F (m/z 441). The oxM residue is also labeled.

Raw File
agg_pep_sort_pos_3

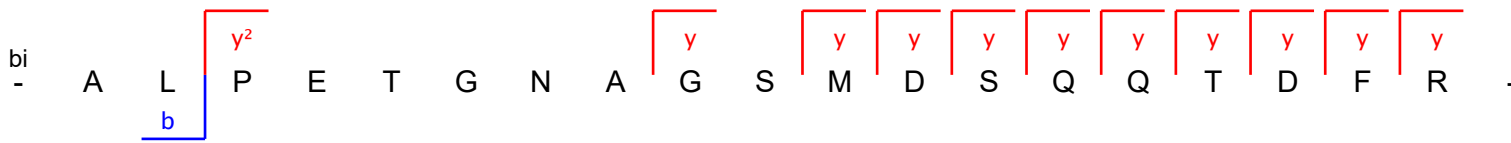
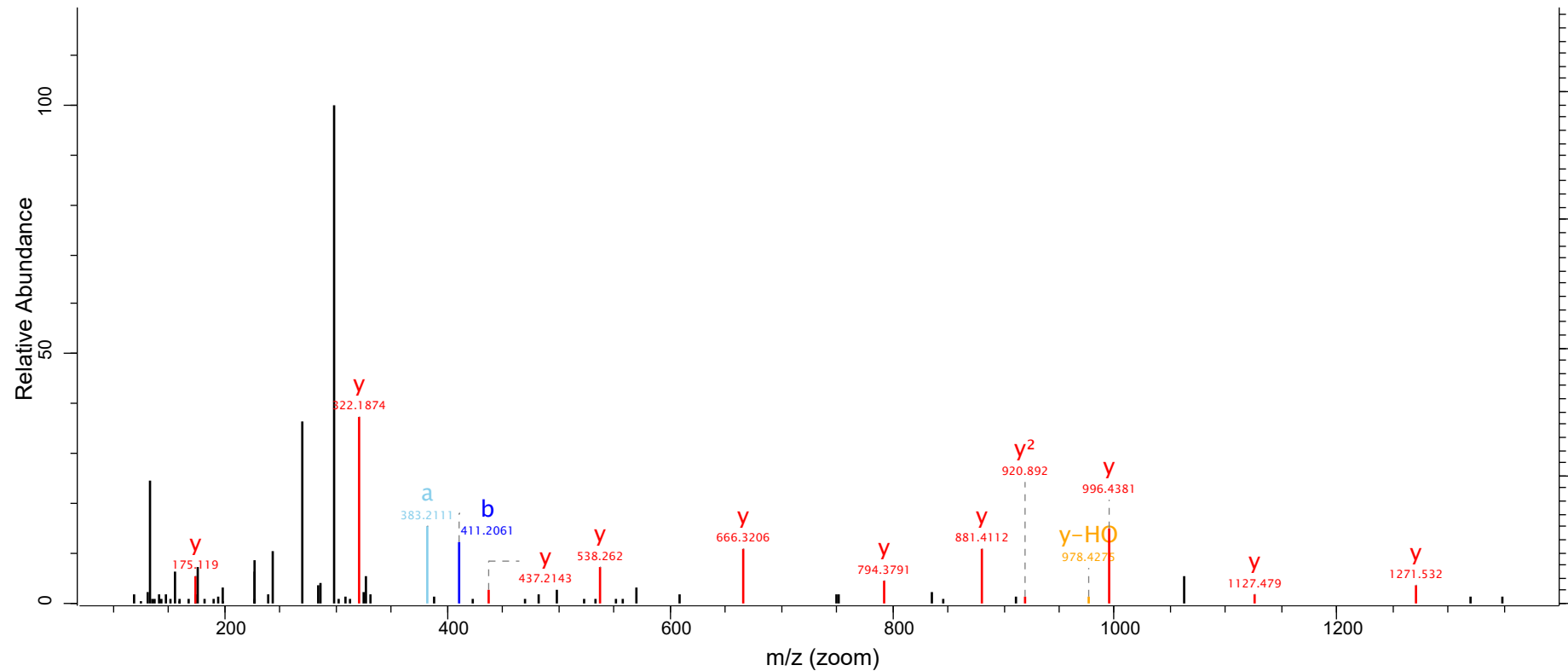
Scan
21412

Method
FTMS; HCD

Score
71.56

m/z
751.33

Gene names
FMNL2



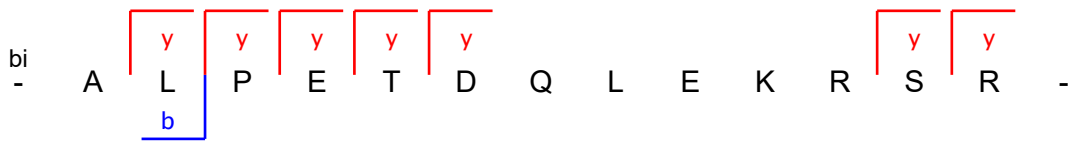
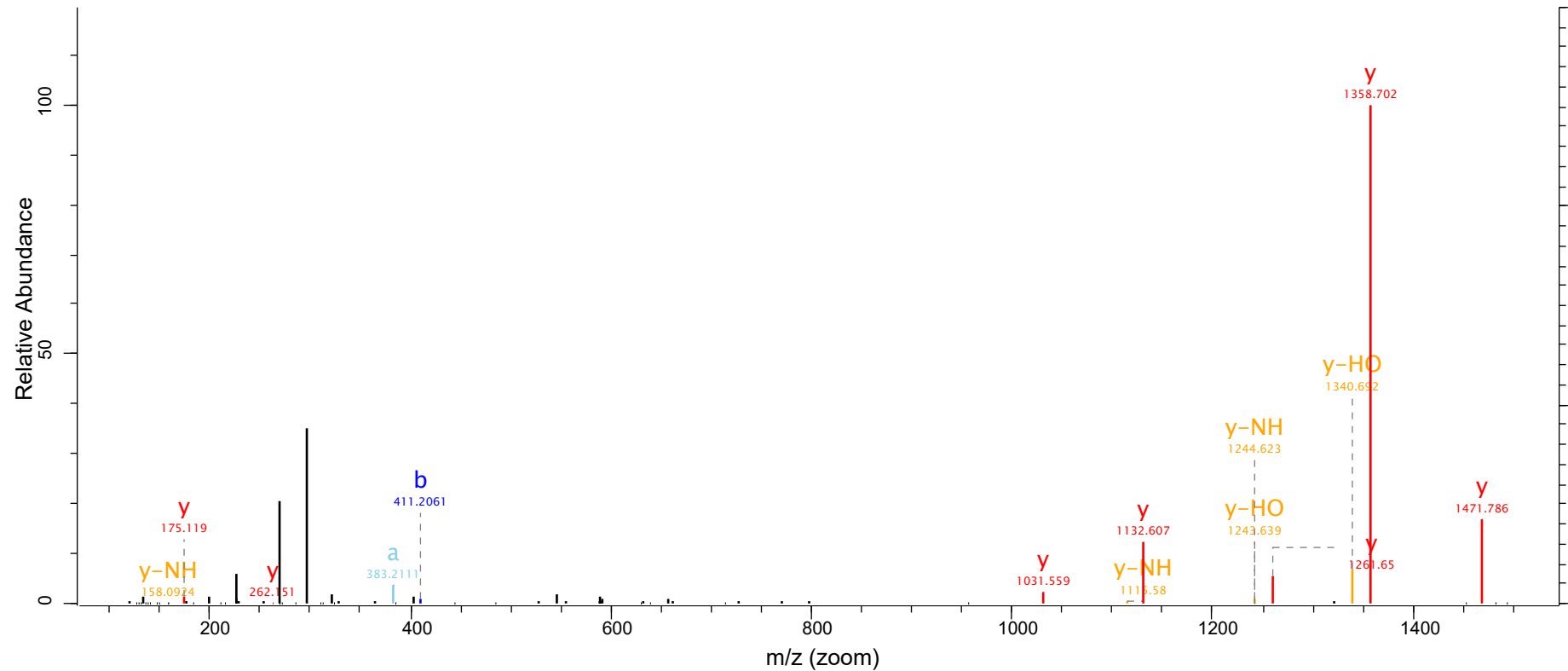
Raw File
agg_pep_sort_pos_3

Scan
16575

Method
FTMS; HCD

Score
65.56

m/z
590.31



Raw File
agg_pep_sort_pos_2

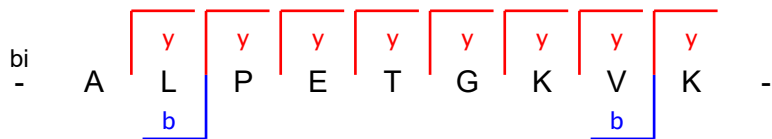
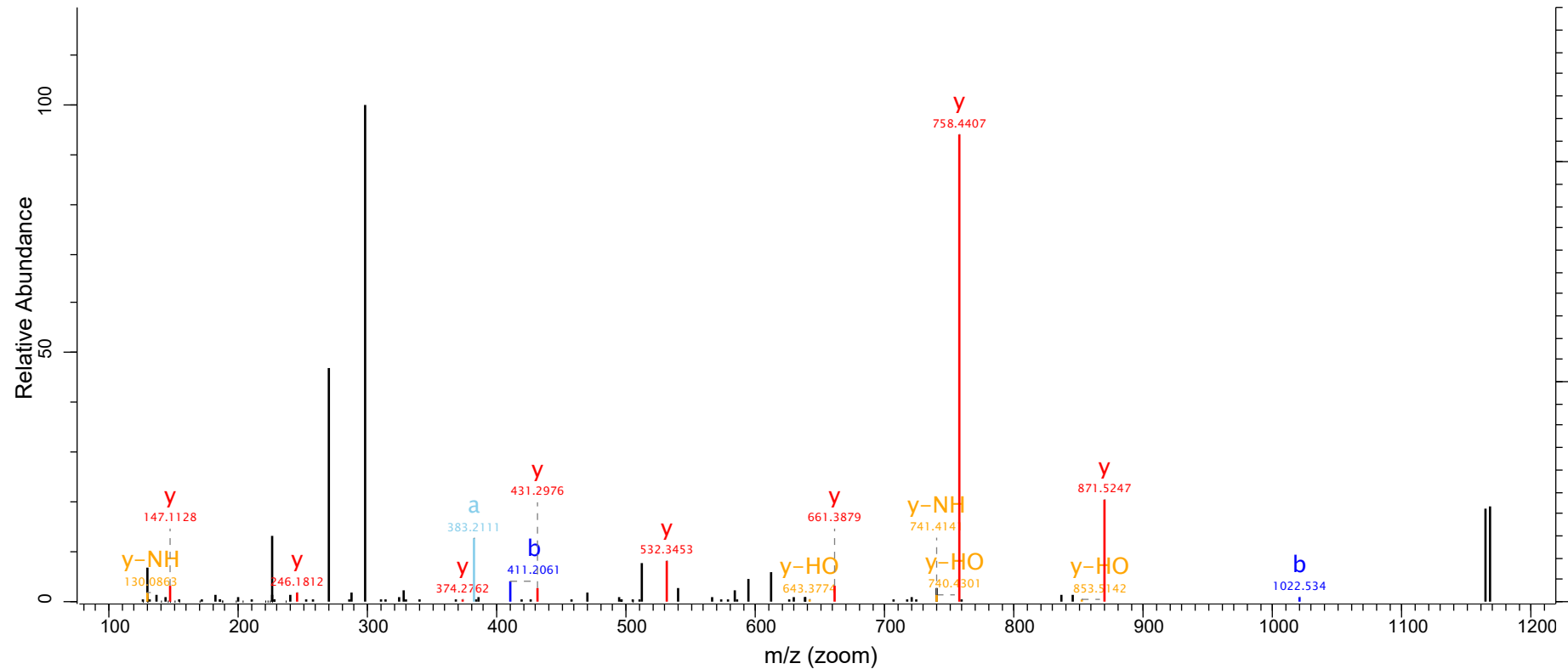
Scan
12916

Method
FTMS; HCD

Score
116.37

m/z
584.82

Gene names
GAPDH



Raw File
agg_pep_sort_pos_1

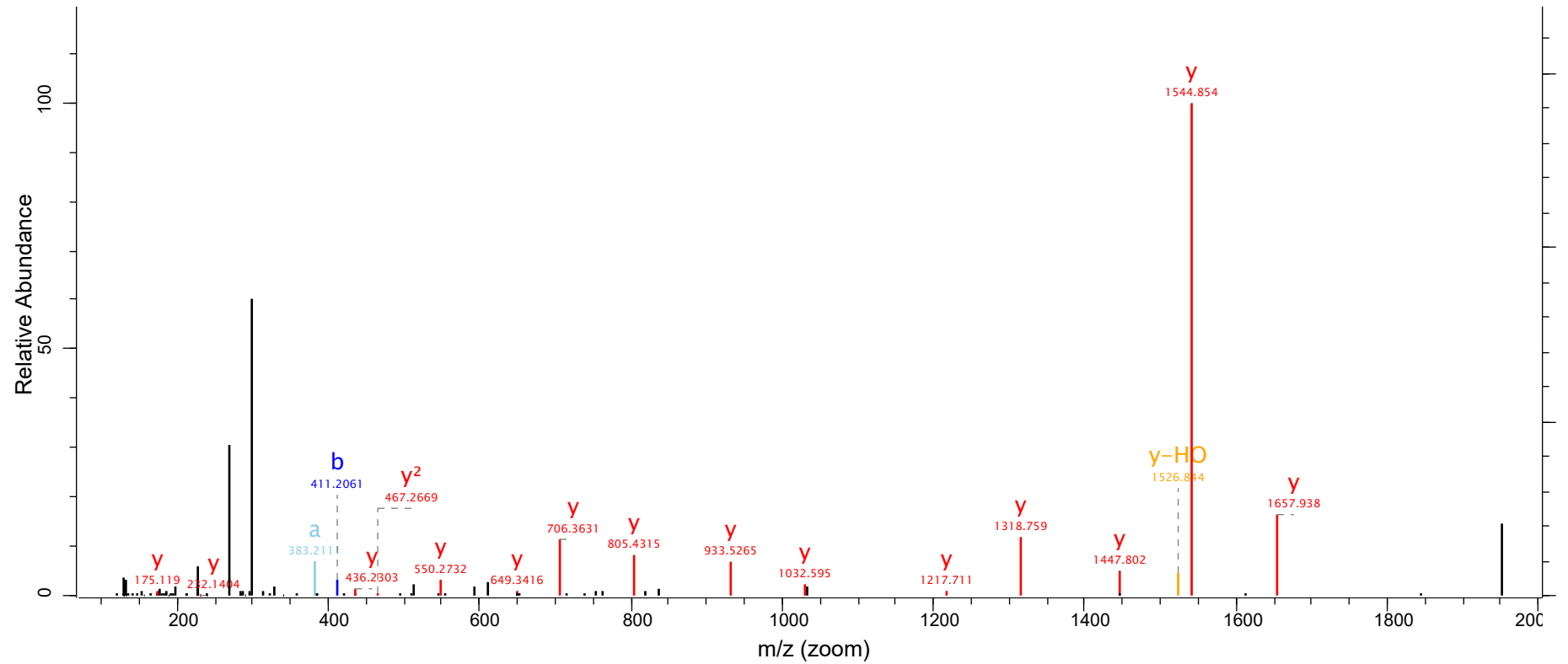
Scan
17445

Method
FTMS; HCD

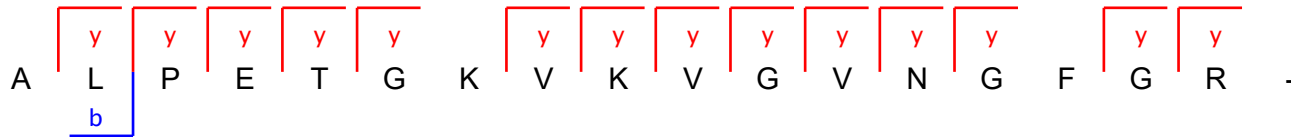
Score
97.84

m/z
652.69

Gene names
GAPDH



bi



Raw File
agg_pep_sort_pos_3

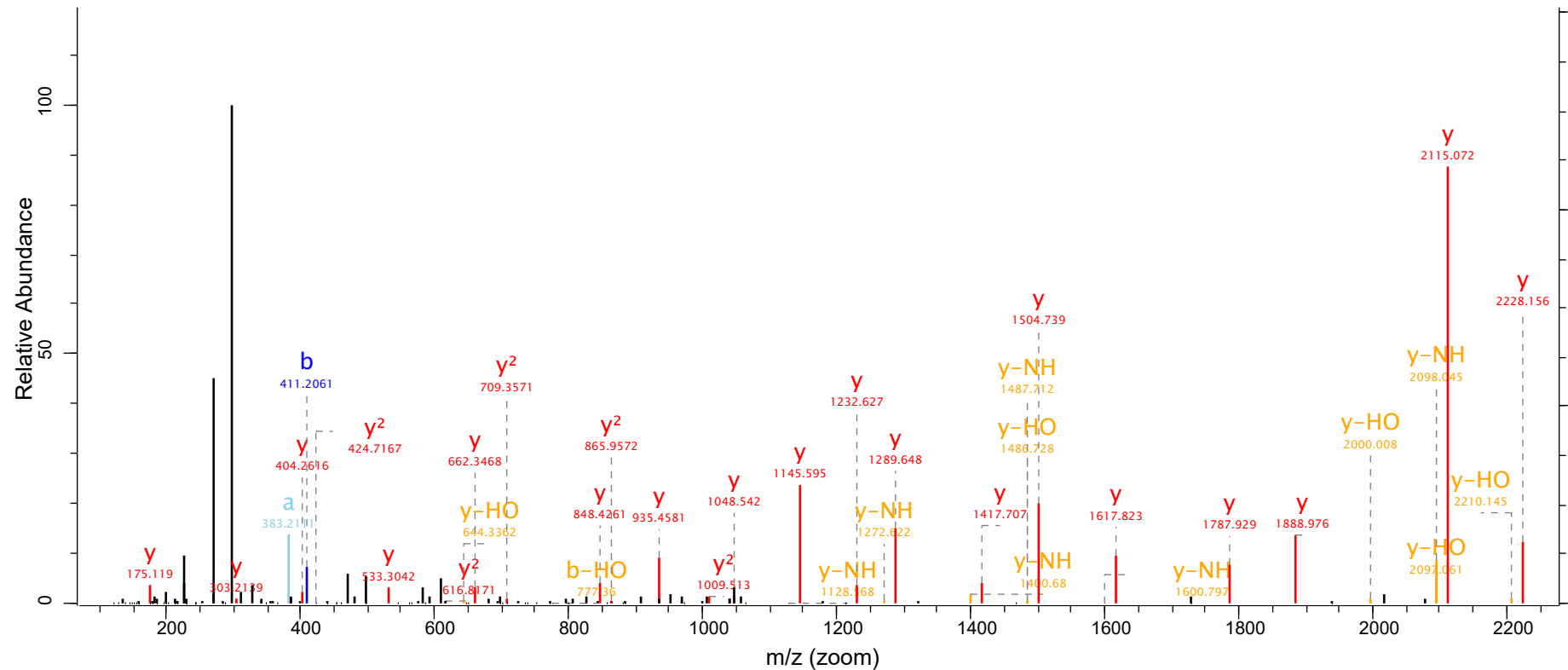
Scan
25685

Method
FTMS; HCD

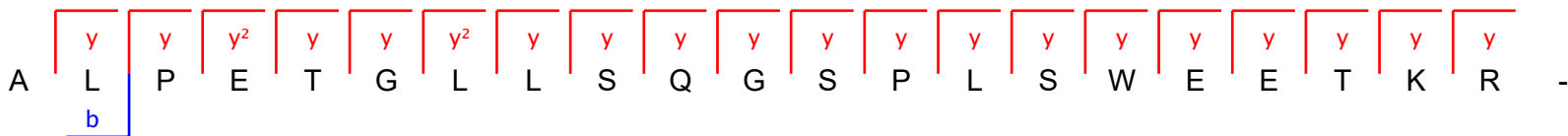
Score
172.84

m/z
842.76

Gene names
GCLC



bi



Raw File
agg_pep_sort_pos_3

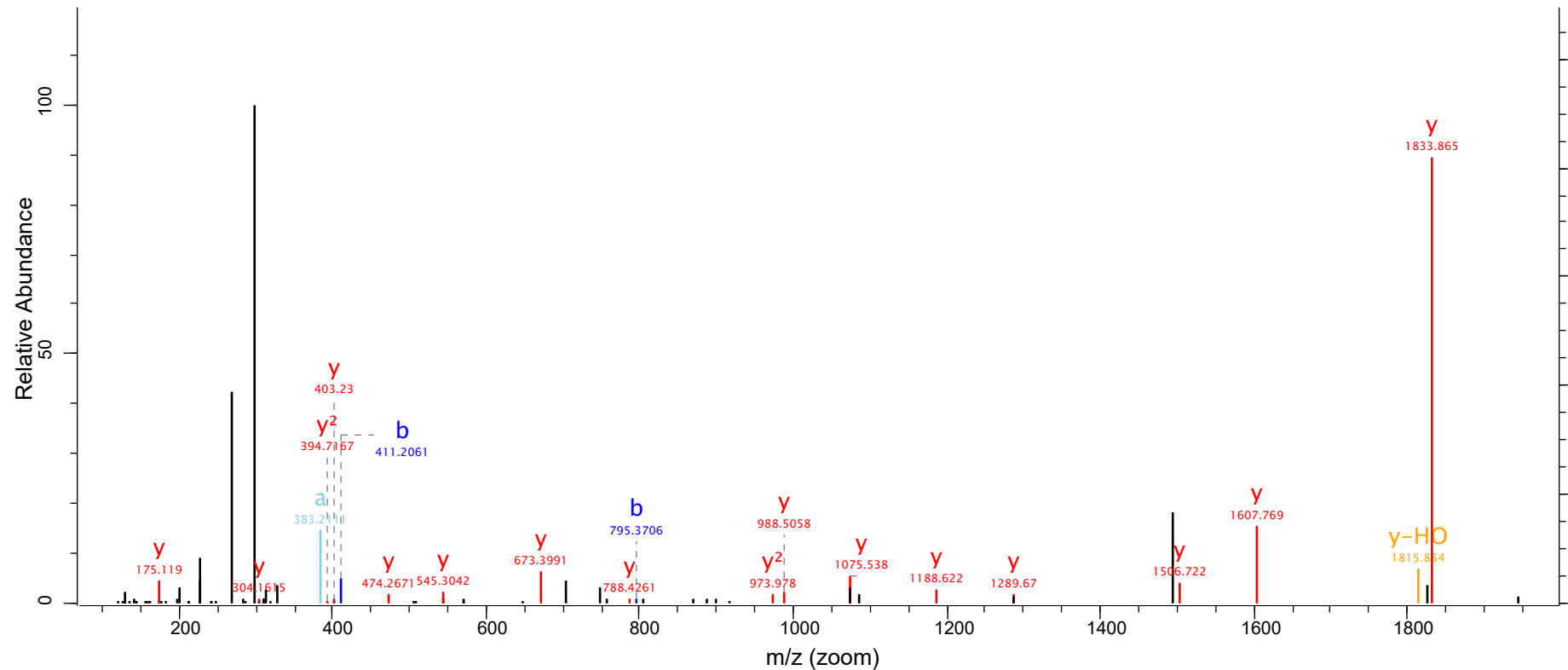
Scan
18017

Method
FTMS; HCD

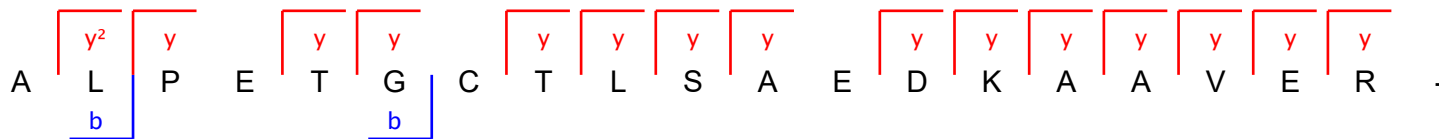
Score
104.61

m/z
749.03

Gene names
GNAI1;GNAI3



bi



Raw File
agg_pep_sort_pos_2

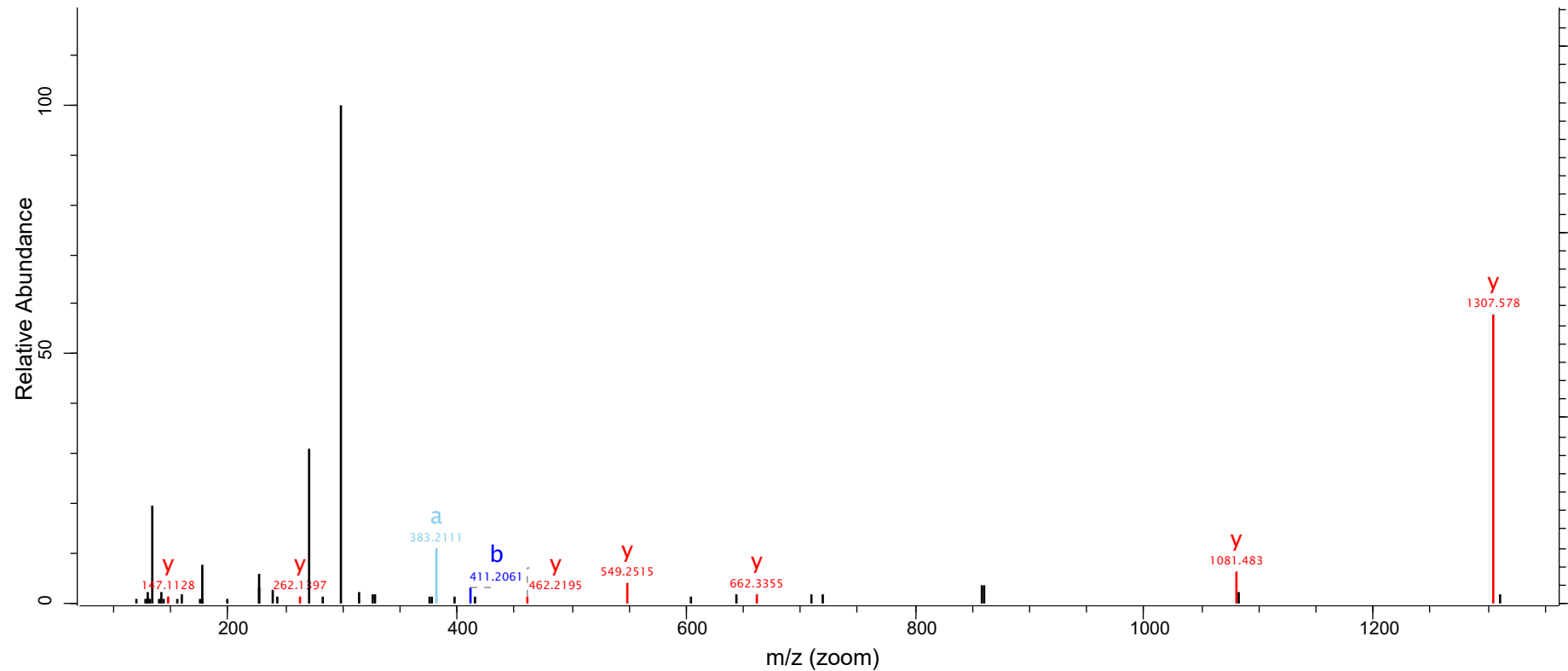
Scan
20403

Method
FTMS; HCD

Score
49.5

m/z
859.89

Gene names
GNAI1;GNAI3



bi
- bi
A L P E T G C T L S A E D K -

Fragmentation diagram showing the sequence A L P E T G C T L S A E D K - with red brackets indicating y-ions and blue brackets indicating b-ions. The b-ion is labeled 'b' and the y-ion is labeled 'y'.

Raw File
agg_pep_sort_pos_3

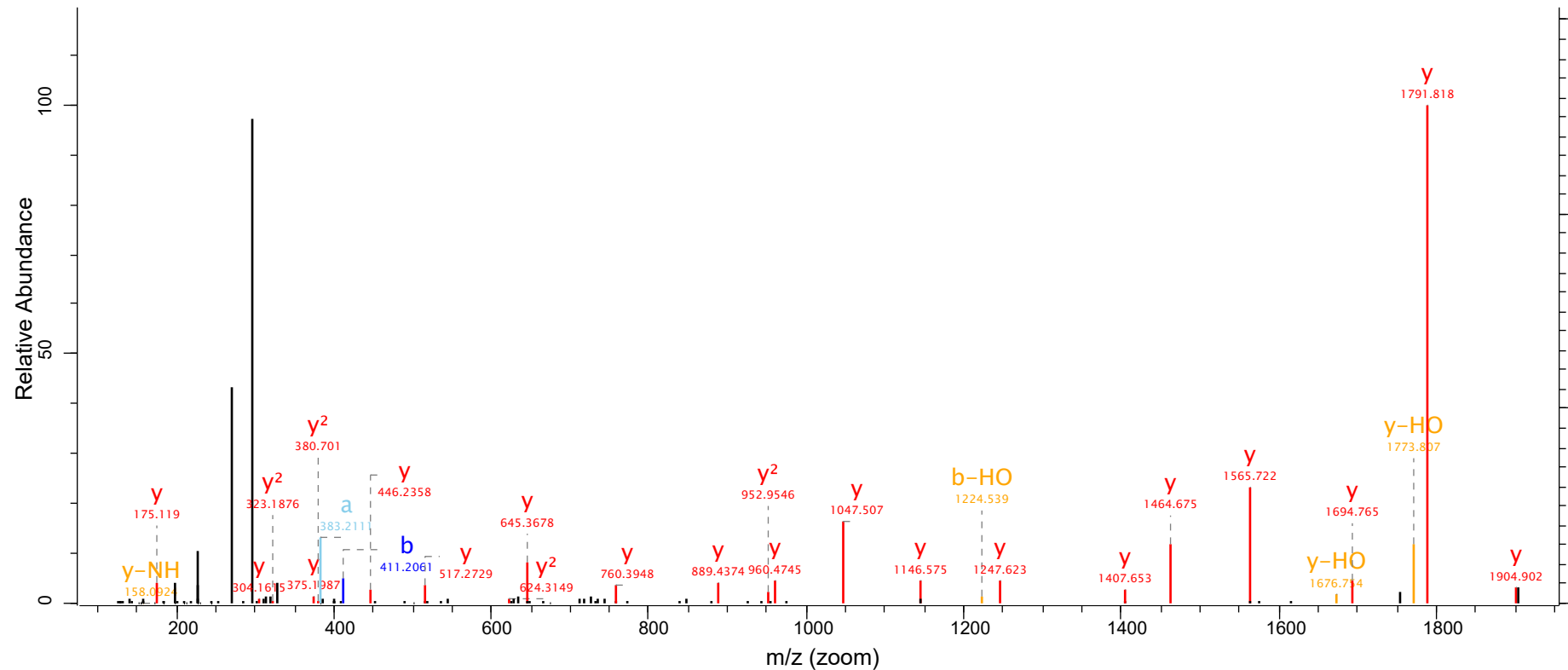
Scan
16044

Method
FTMS; HCD

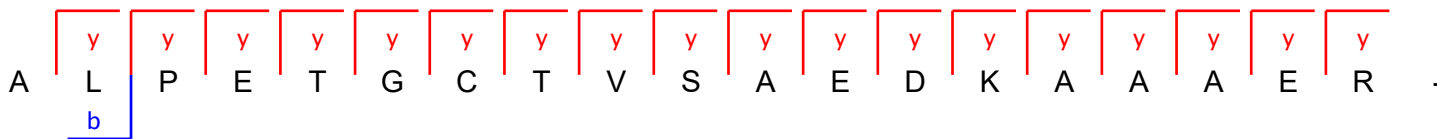
Score
141.86

m/z
735.01

Gene names
GNAI2



bi



Raw File
agg_pep_sort_pos_1

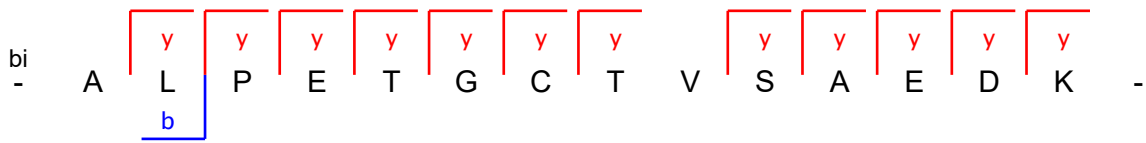
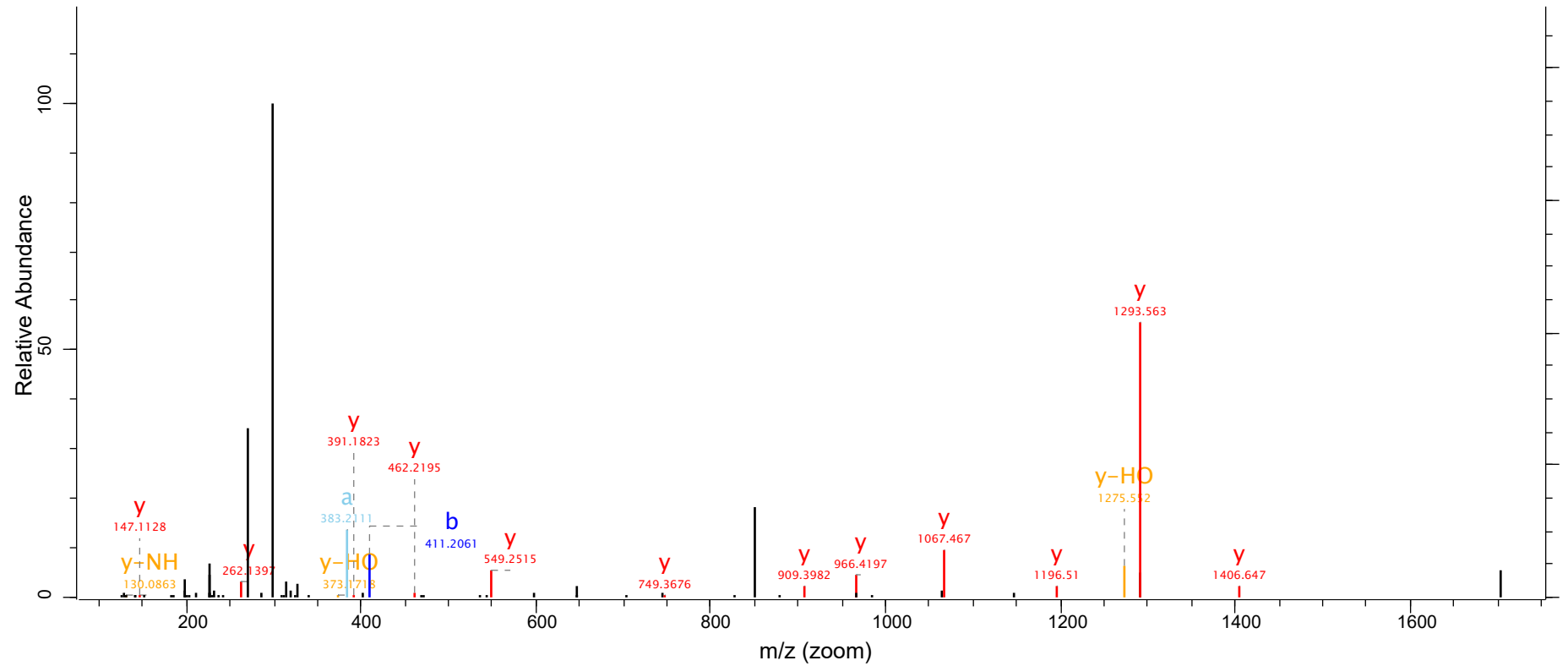
Scan
19277

Method
FTMS; HCD

Score
85.29

m/z
852.38

Gene names
GNAI2



Raw File
agg_pep_sort_pos_2

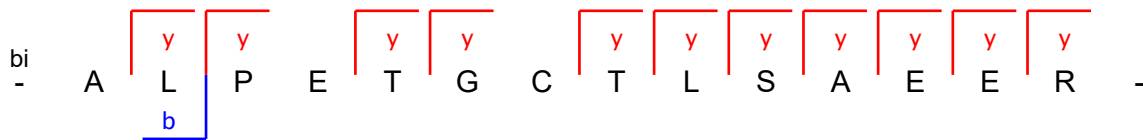
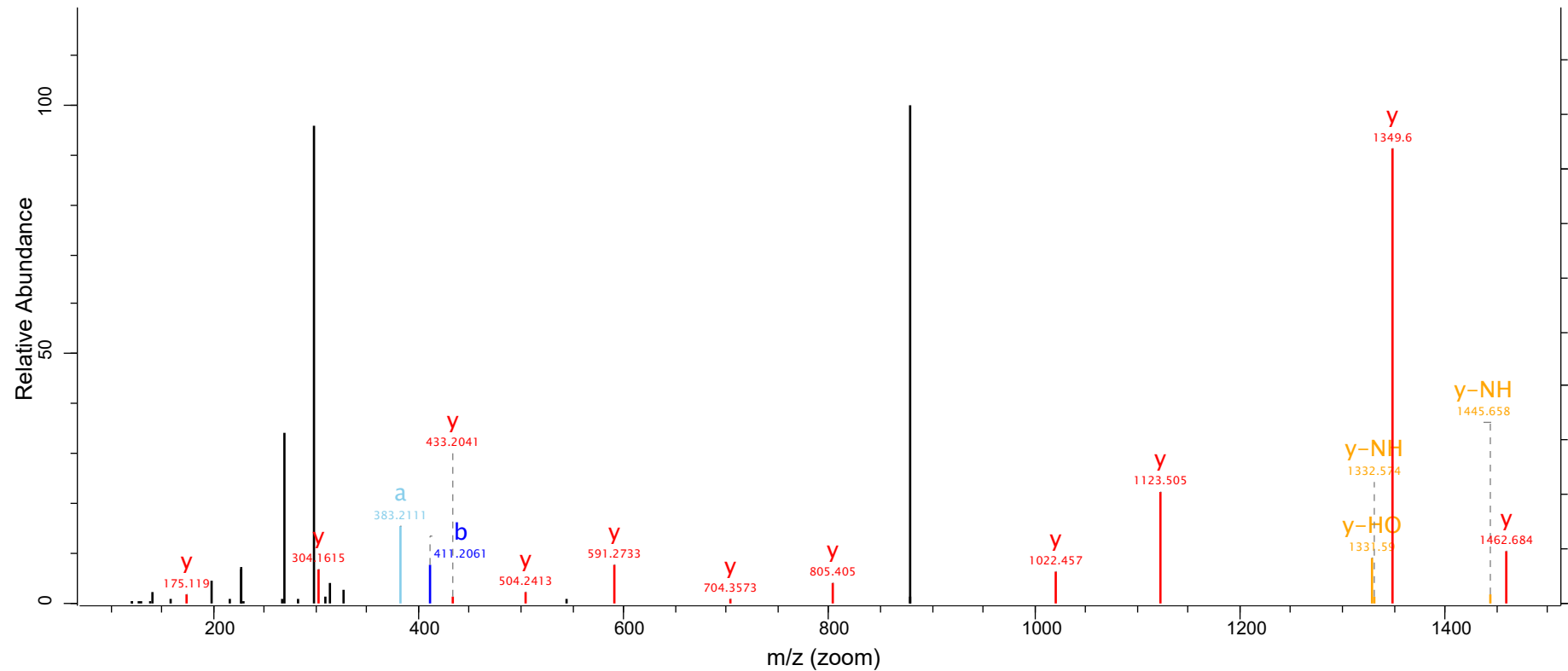
Scan
20634

Method
FTMS; HCD

Score
113.63

m/z
880.9

Gene names
GNAO1



Raw File
agg_pep_sort_pos_1

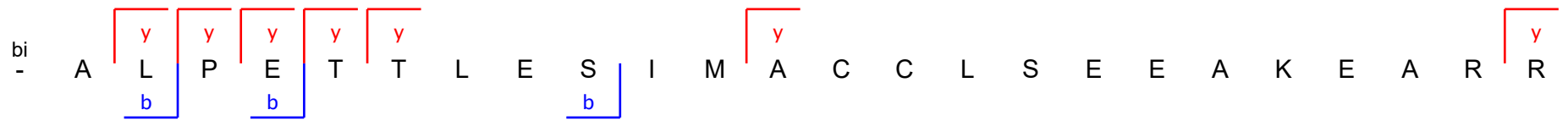
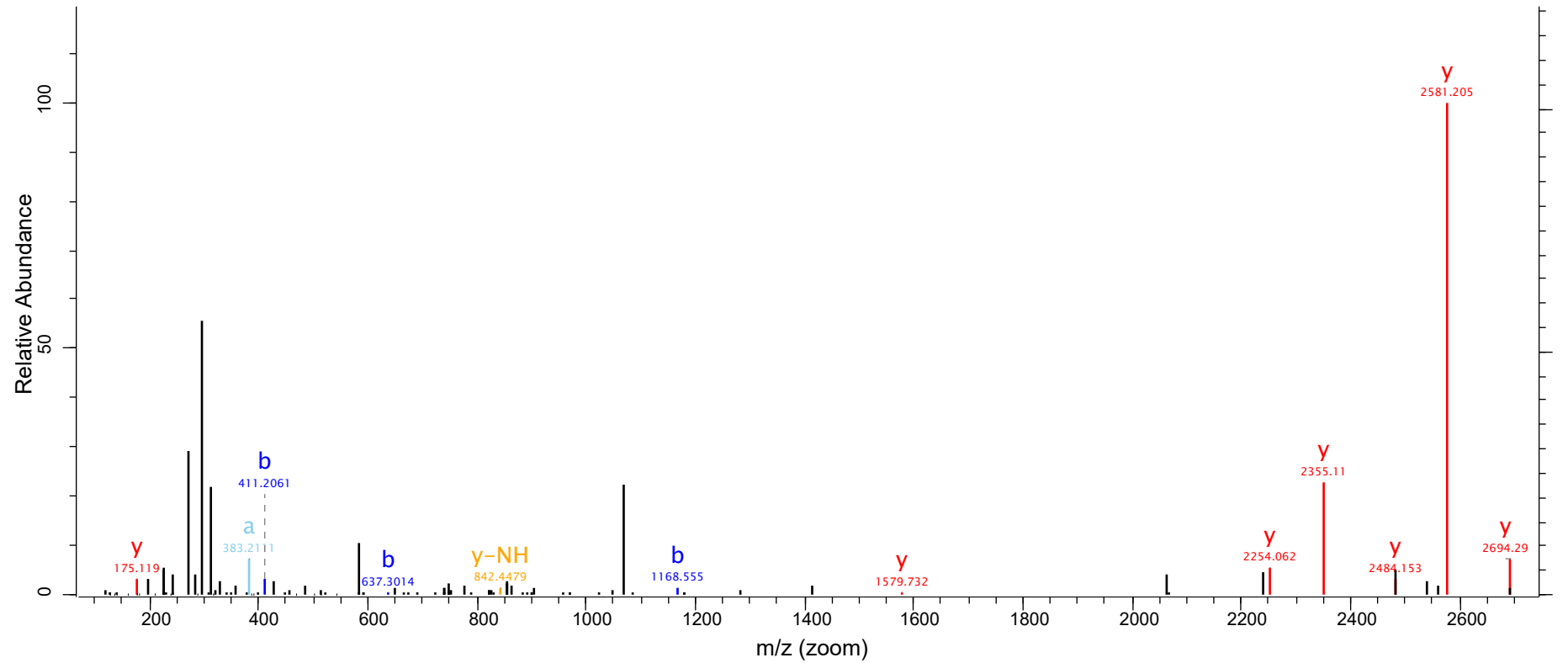
Scan
17320

Method
FTMS; HCD

Score
42.32

m/z
749.11

Gene names
GNAQ



Raw File
agg_pep_sort_pos_1

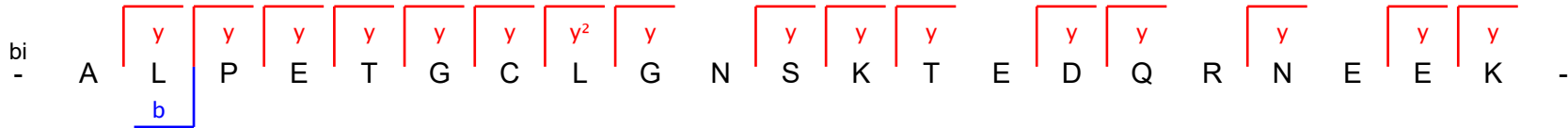
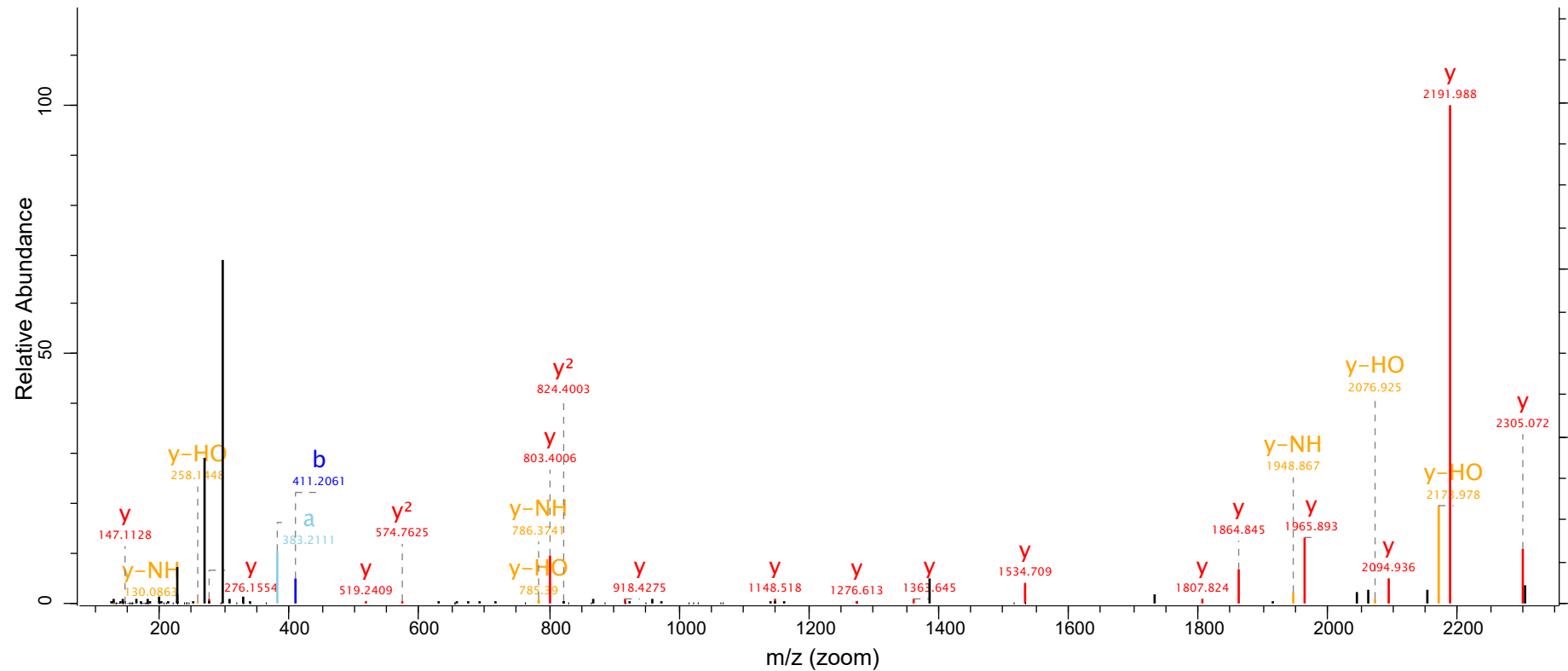
Scan
13691

Method
FTMS; HCD

Score
118.52

m/z
868.4

Gene names
GNAS



Raw File
agg_pep_sort_pos_3

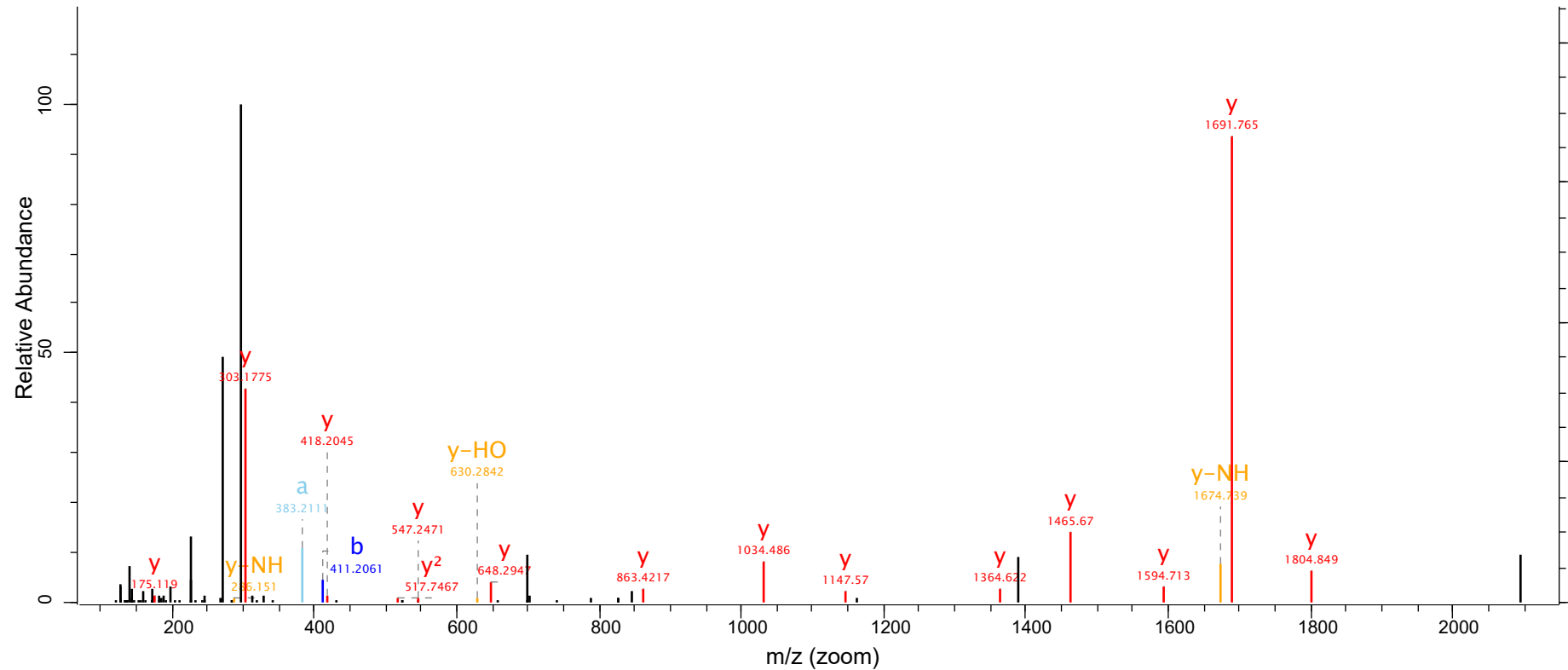
Scan
16056

Method
FTMS; HCD

Score
98.51

m/z
701.33

Gene names
GNAS



bi



Raw File
agg_pep_sort_pos_3

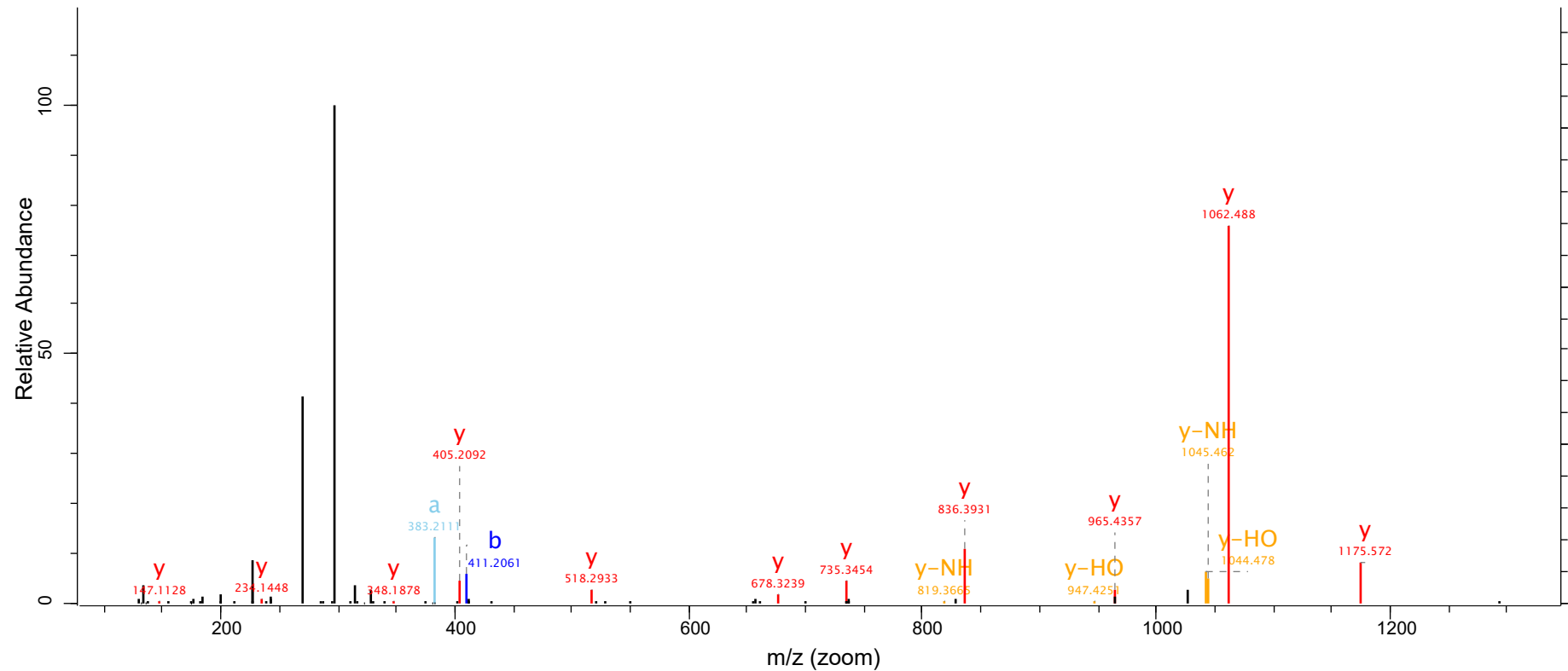
Scan
19653

Method
FTMS; HCD

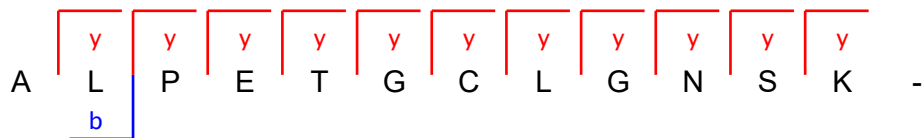
Score
88.06

m/z
736.85

Gene names
GNAS



bi



Raw File
agg_pep_sort_pos_3

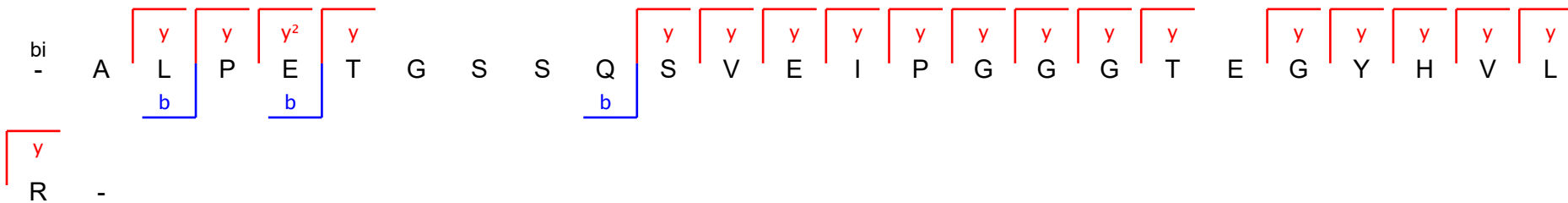
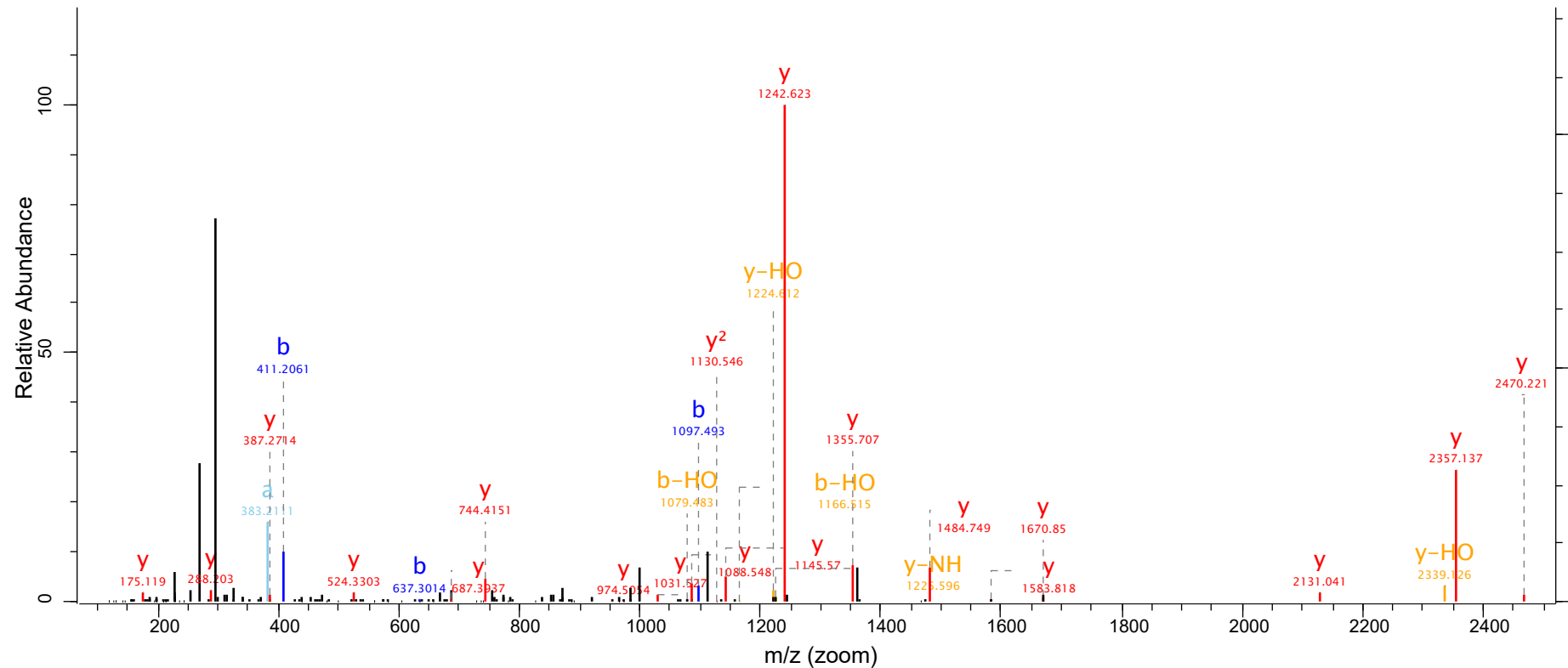
Scan
21150

Method
FTMS; HCD

Score
86.79

m/z
923.45

Gene names
GORASP2



Raw File
agg_pep_sort_pos_1

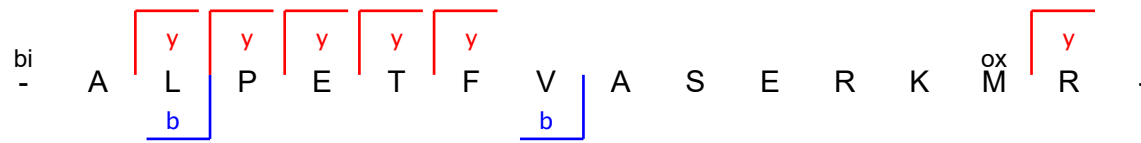
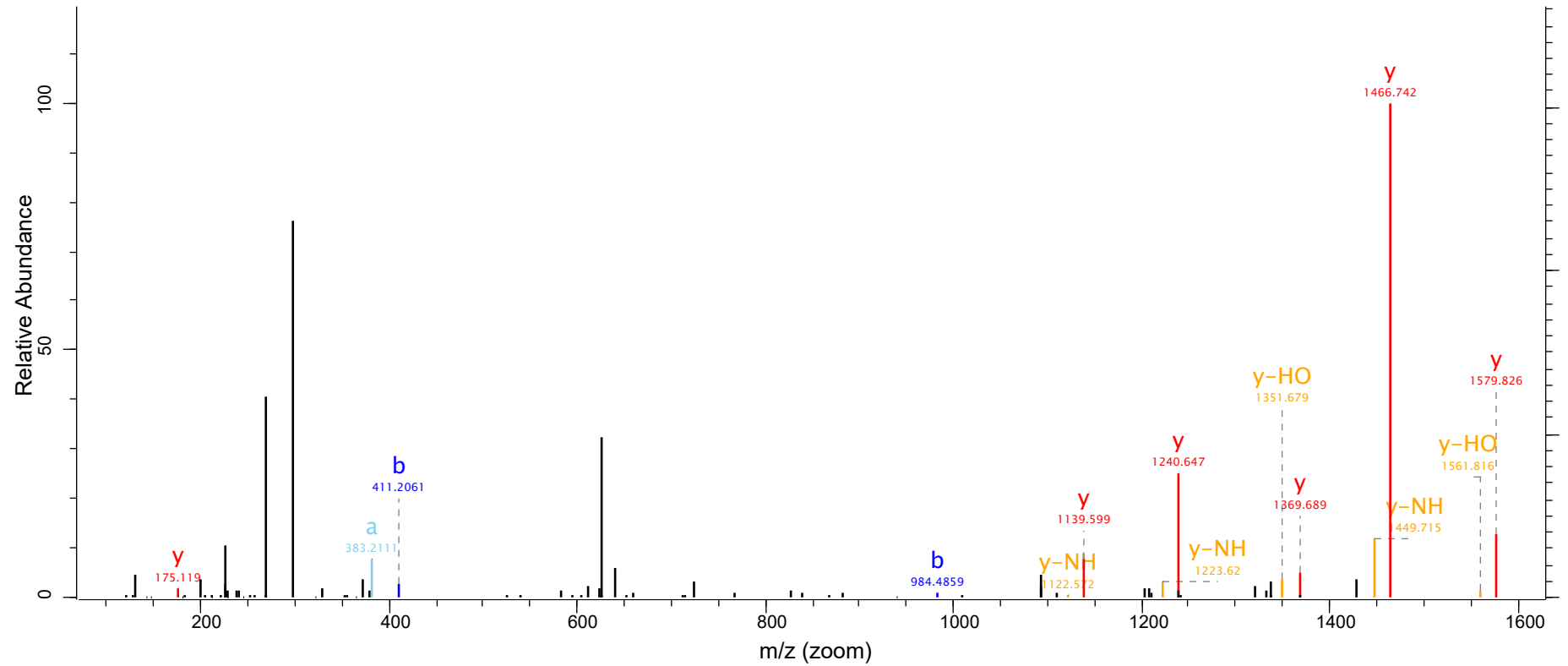
Scan
17822

Method
FTMS; HCD

Score
82.54

m/z
626.32

Gene names
GPC5B



Raw File
agg_pep_sort_pos_1

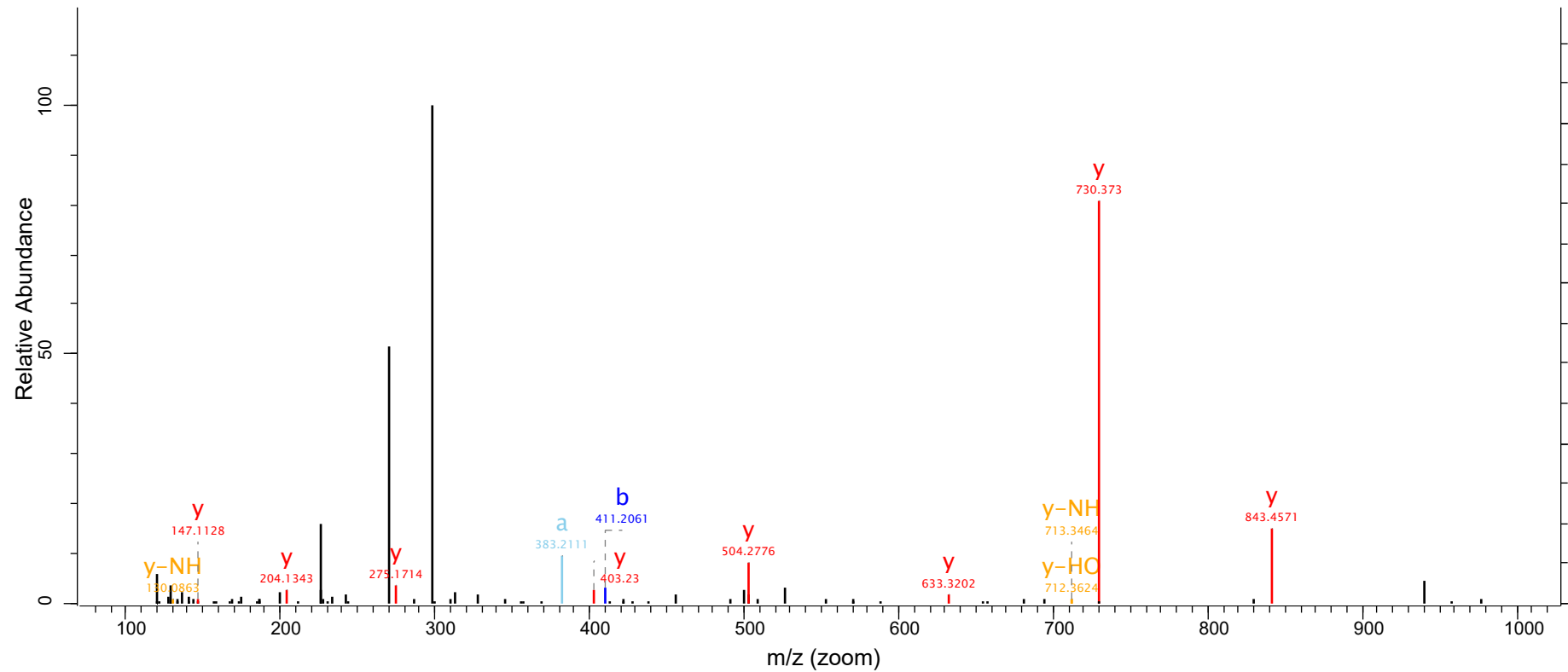
Scan
17337

Method
FTMS; HCD

Score
83.21

m/z
571.29

Gene names
GSTZ1



Raw File
agg_pep_sort_pos_1

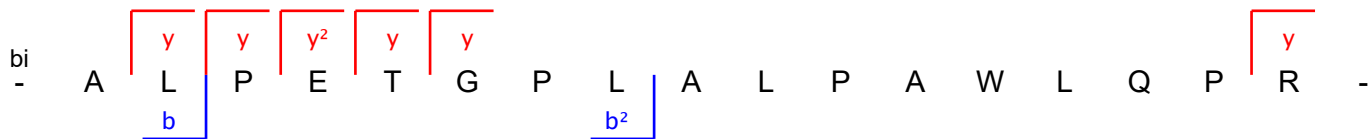
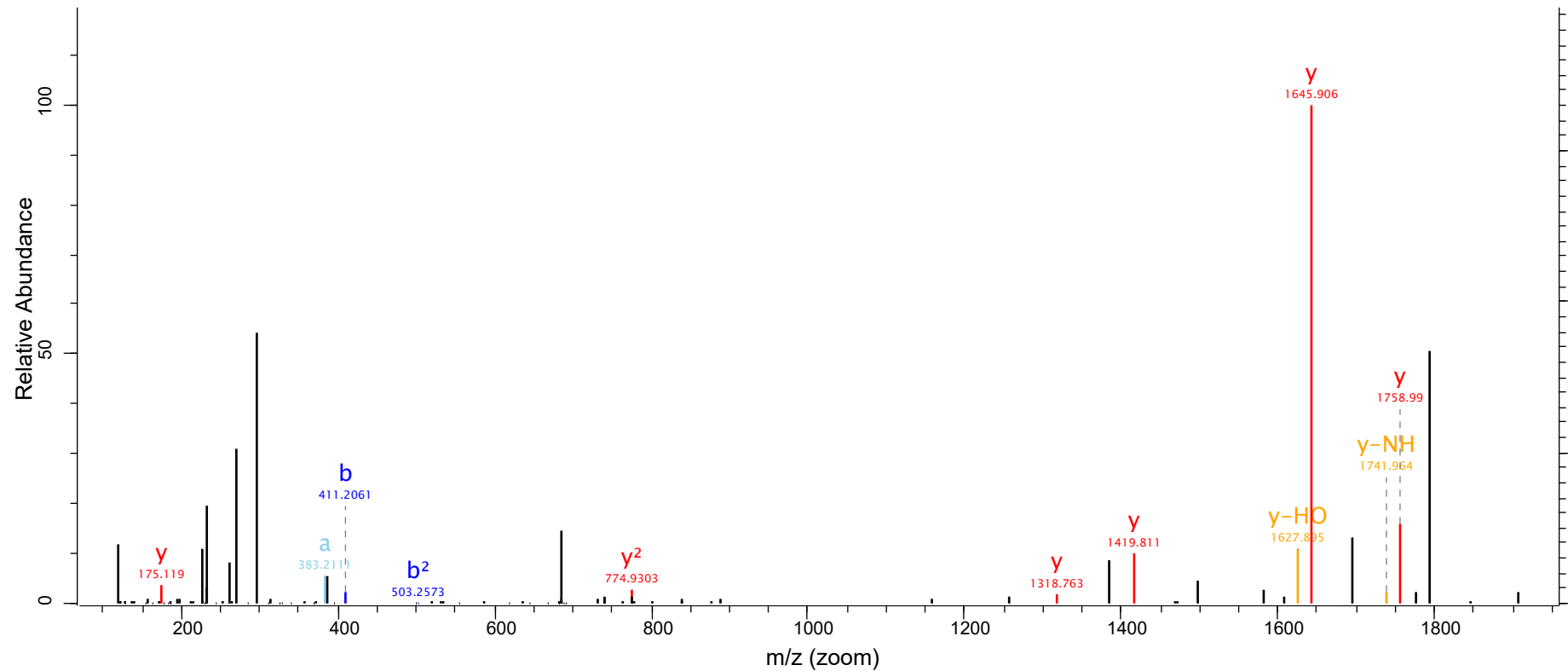
Scan
18957

Method
FTMS; HCD

Score
44.81

m/z
686.37

Gene names
HACD4



Raw File
agg_pep_sort_pos_1

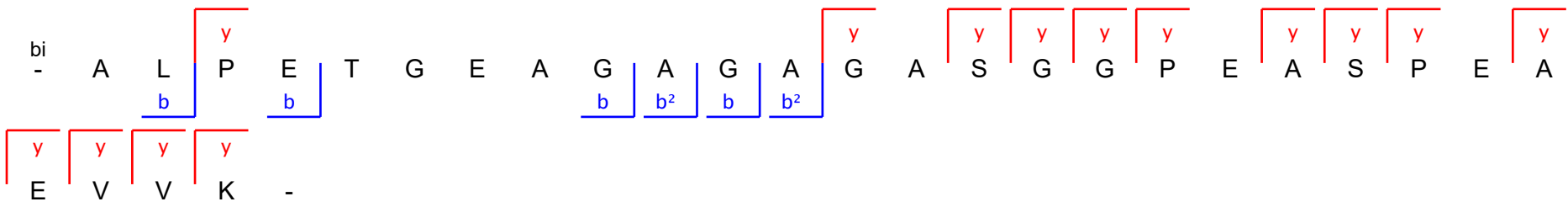
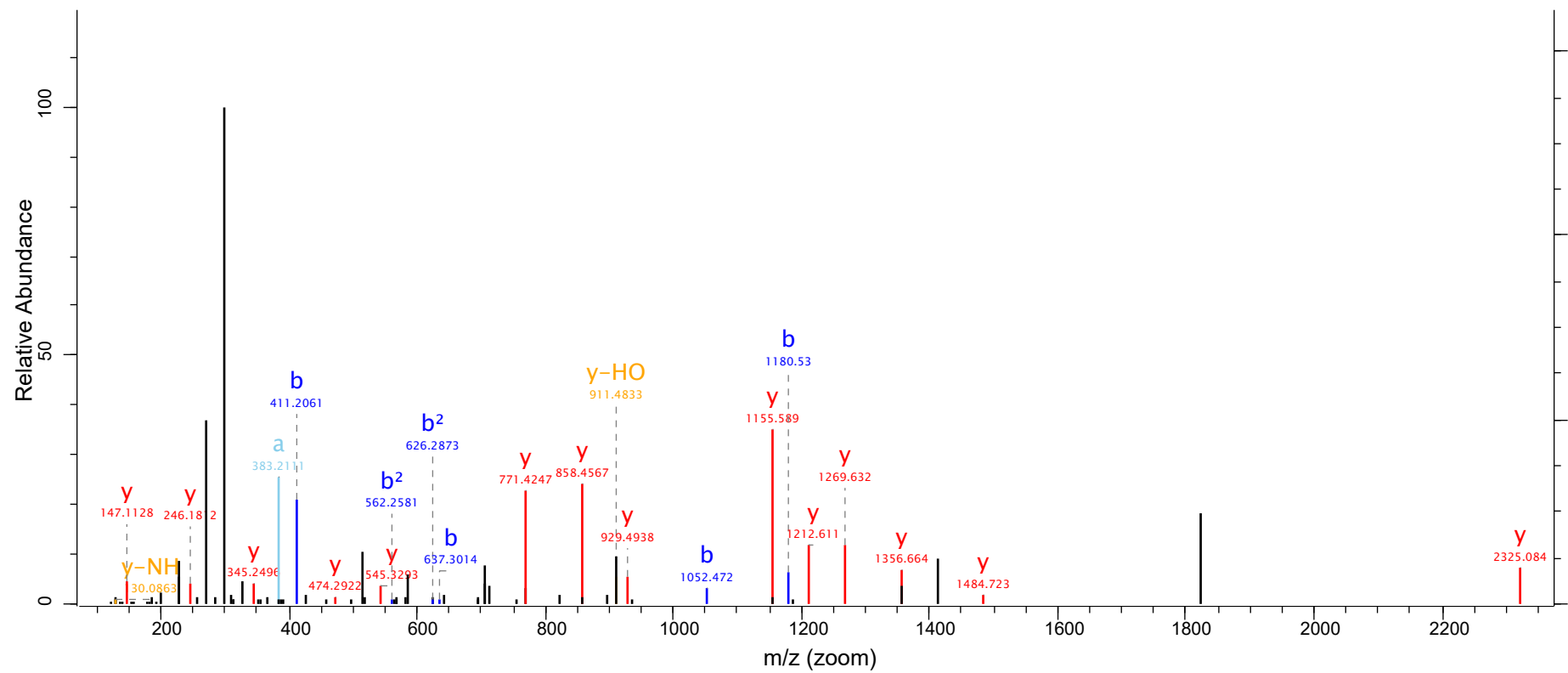
Scan
21429

Method
FTMS; HCD

Score
65.78

m/z
912.77

Gene names
HGHI



Raw File
agg_pep_sort_pos_2

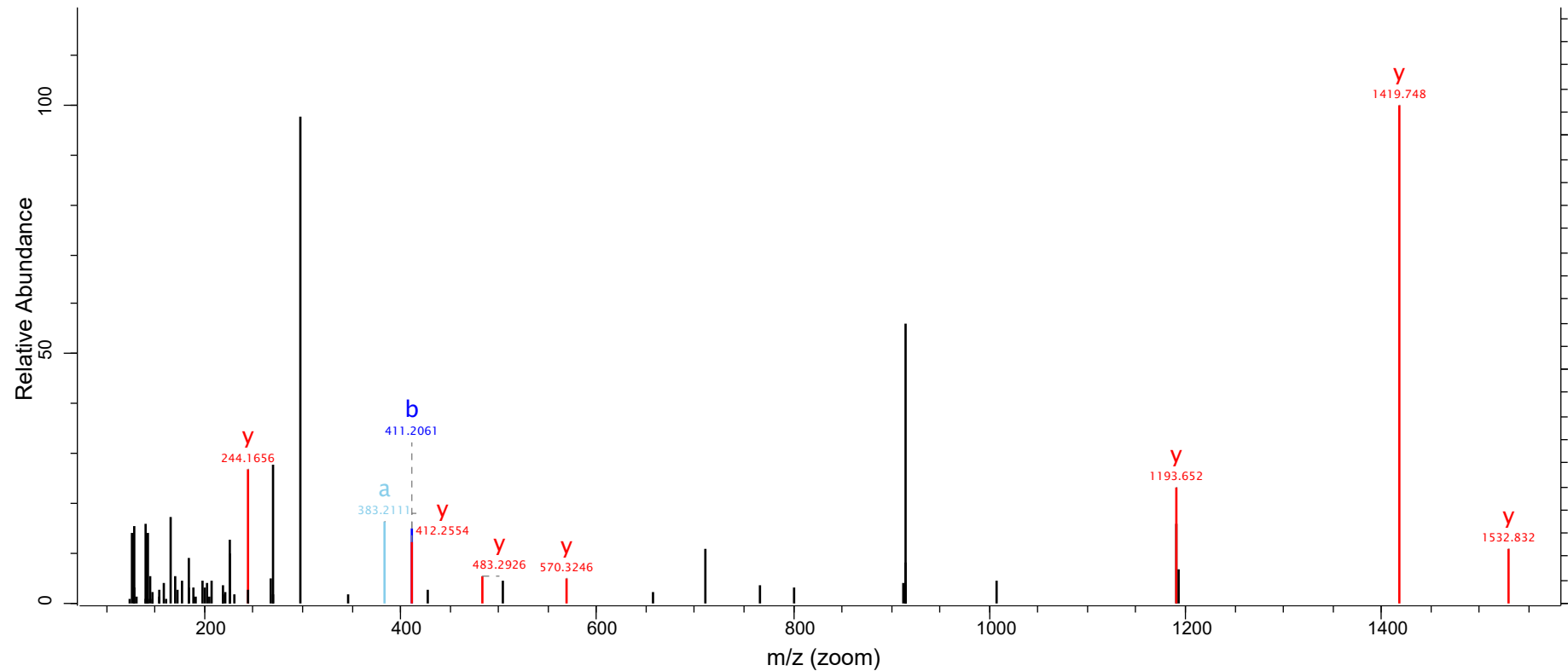
Scan
14609

Method
FTMS; HCD

Score
55.4

m/z
915.98

Gene names
HIST2H2BE; HIST1H2BC; H2BFS; HIST1H2BJ; HIST1H2BK



Raw File
agg_pep_sort_pos_2

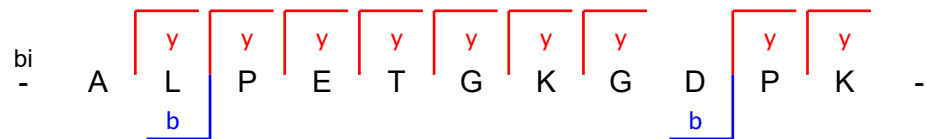
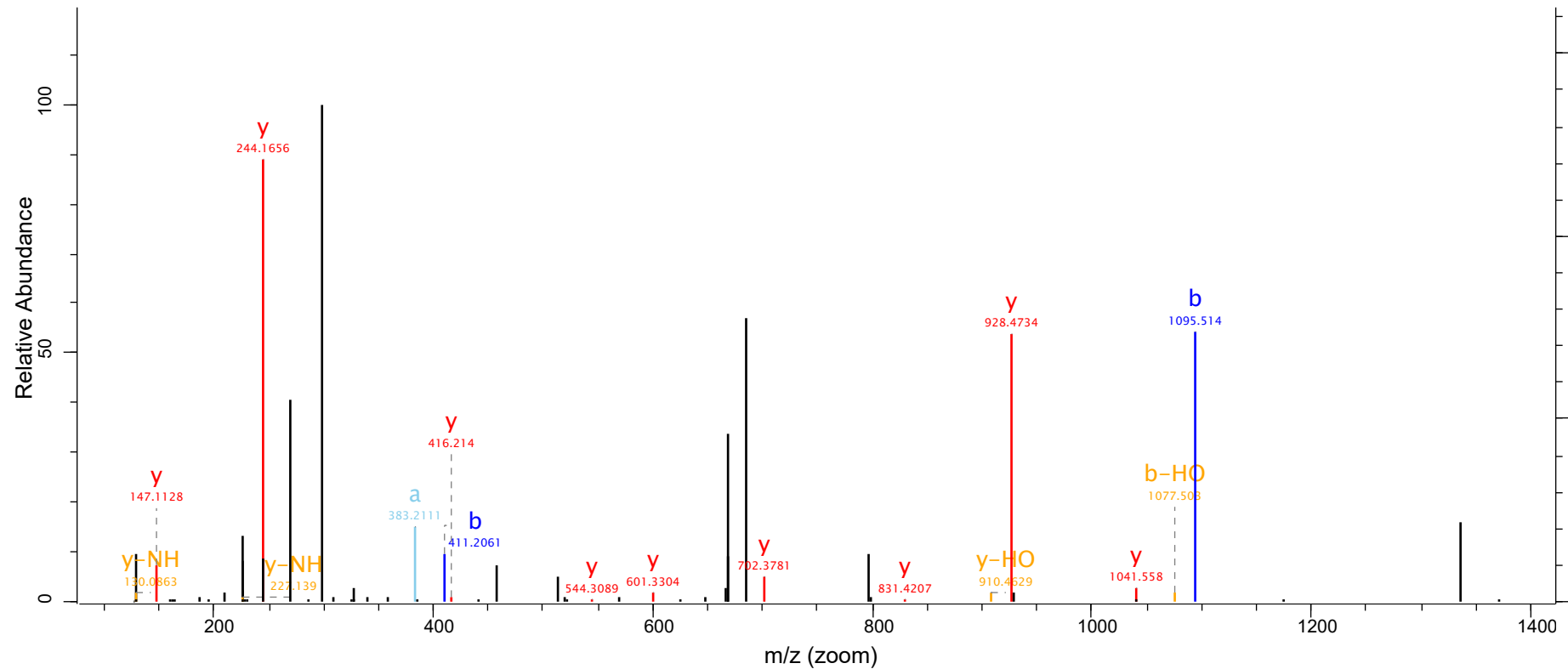
Scan
12260

Method
FTMS; HCD

Score
99.82

m/z
669.84

Gene names
HMGB1;HMGB1P1



Raw File
agg_pep_sort_pos_1

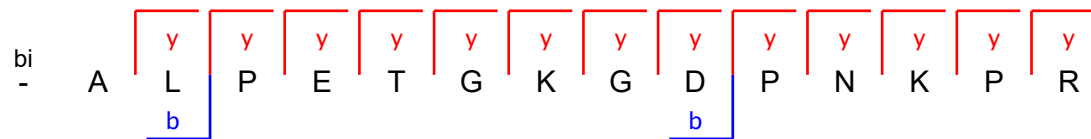
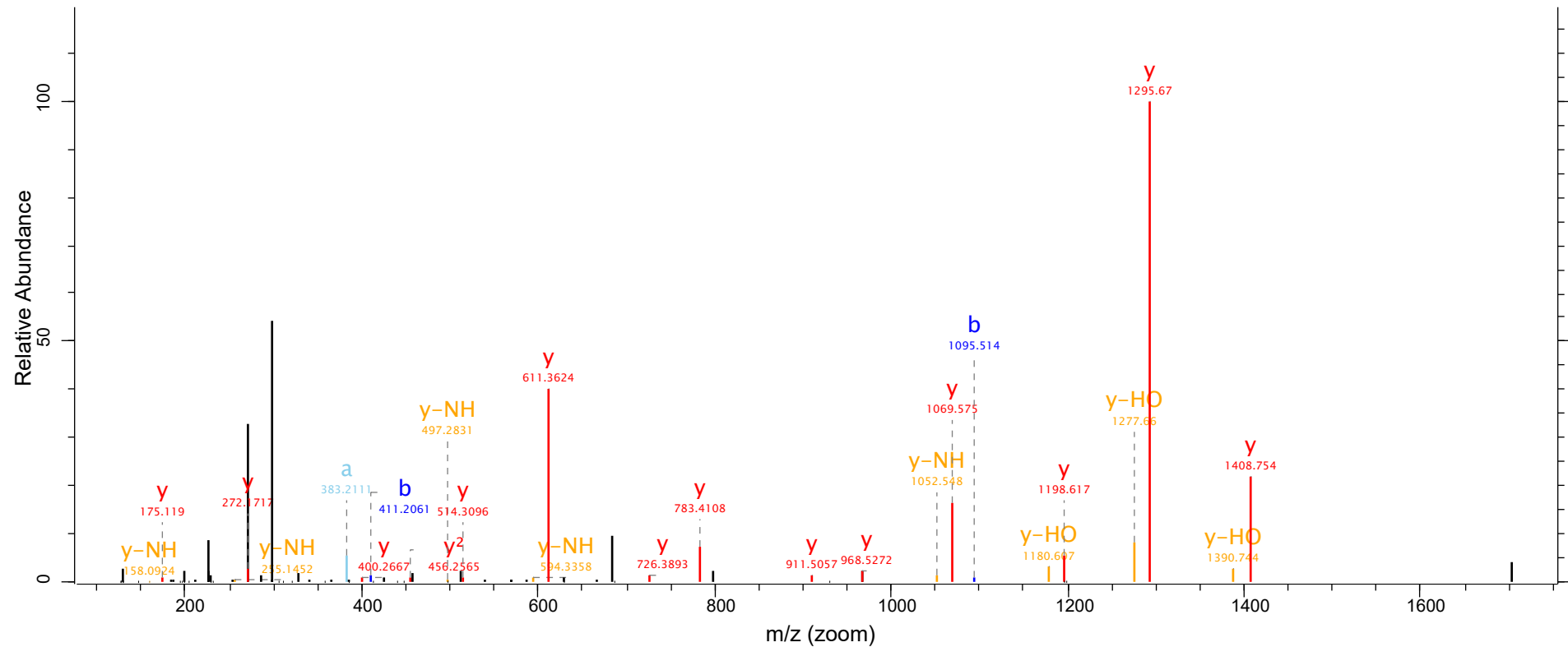
Scan
10291

Method
FTMS; HCD

Score
143.98

m/z
569.3

Gene names
HMGB2



Raw File
agg_pep_sort_pos_3

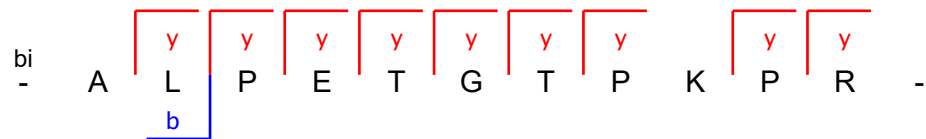
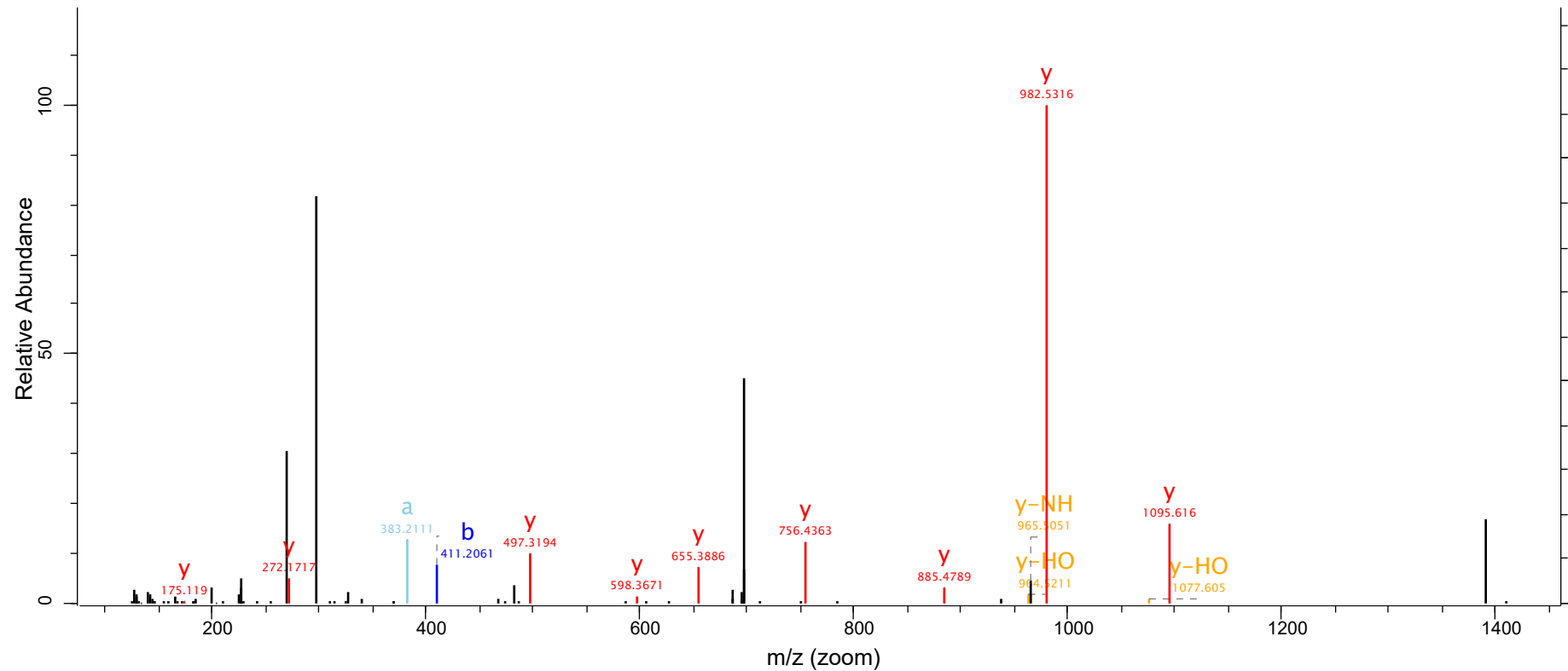
Scan
13735

Method
FTMS; HCD

Score
84.21

m/z
697.37

Gene names
IRF3



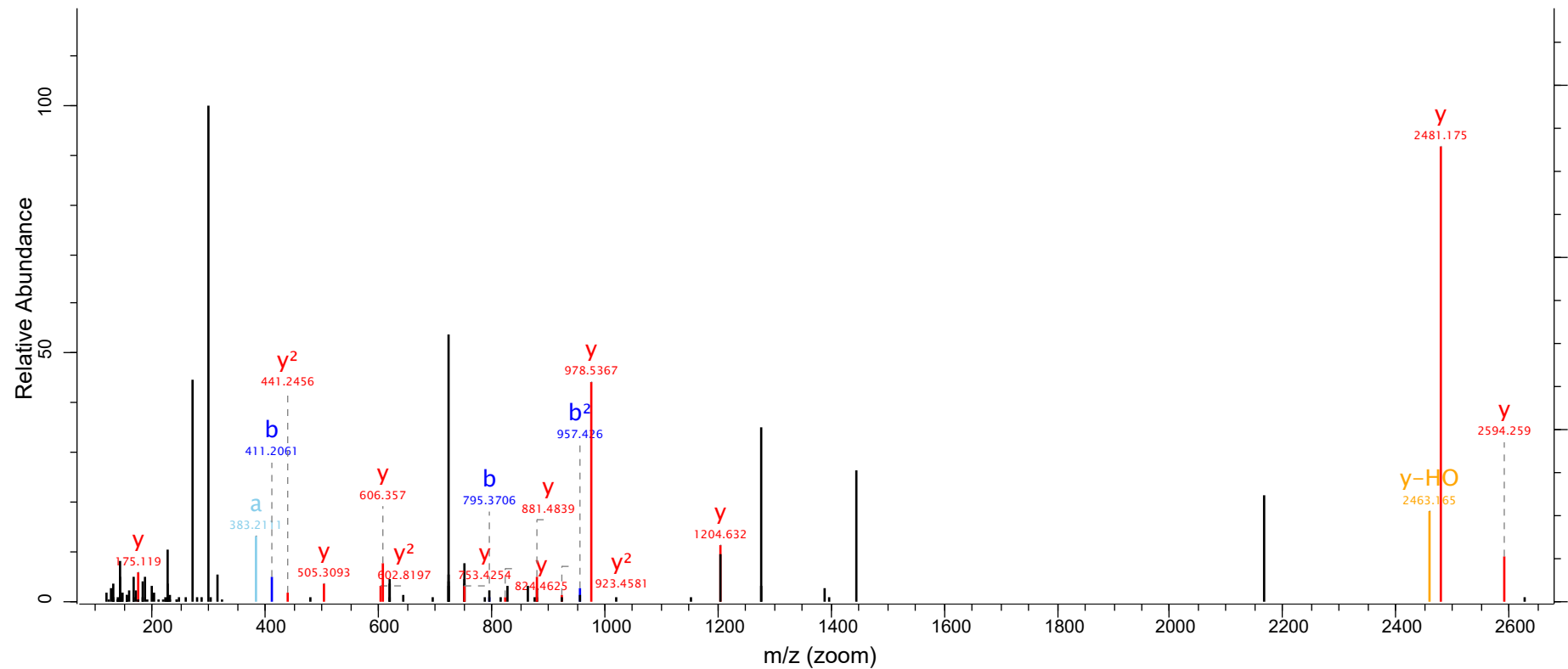
Raw File
agg_pep_sort_pos_3

Scan
13392

Method
FTMS; HCD

Score
66.38

m/z
724.1



Raw File
agg_pep_sort_pos_1

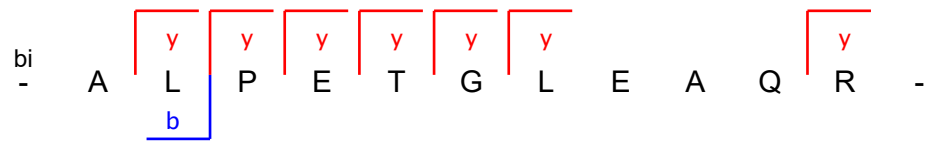
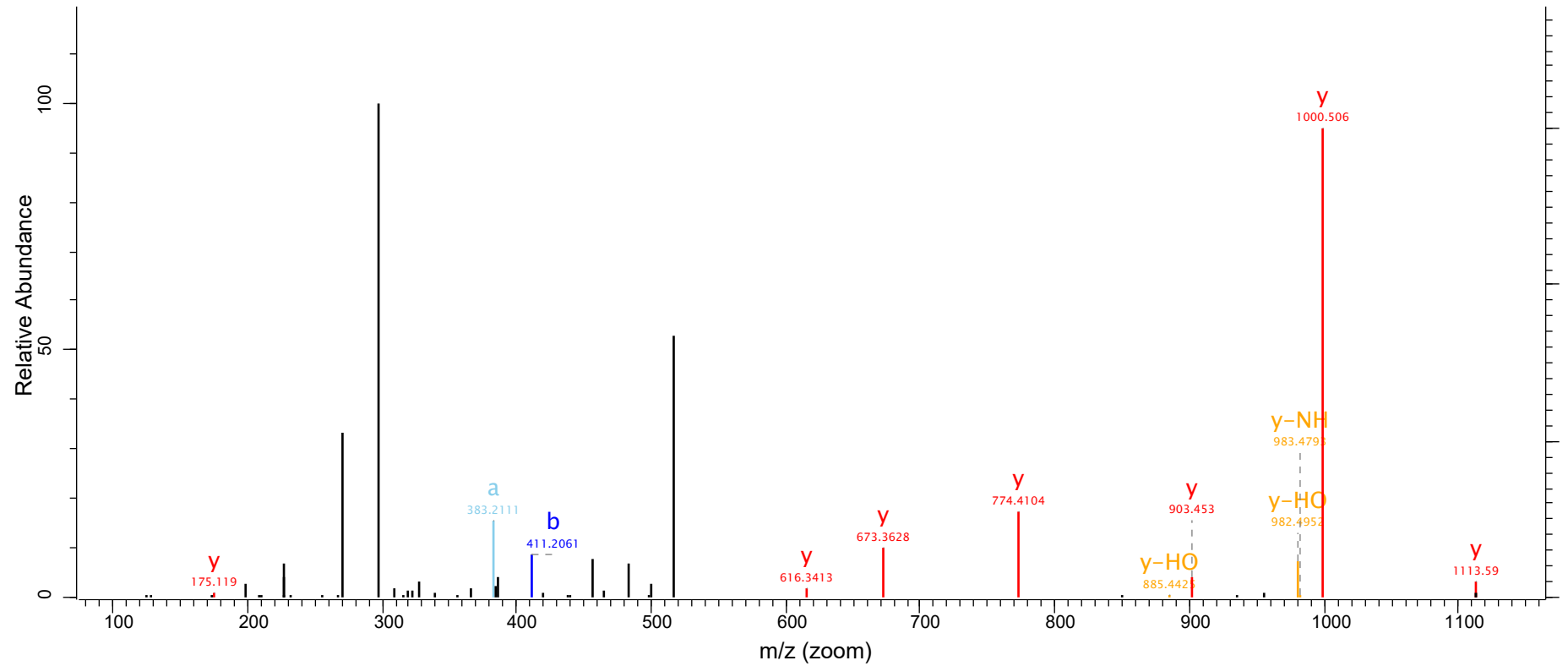
Scan
20266

Method
FTMS; HCD

Score
92.06

m/z
705.86

Gene names
KIF7



Raw File
agg_pep_sort_pos_2

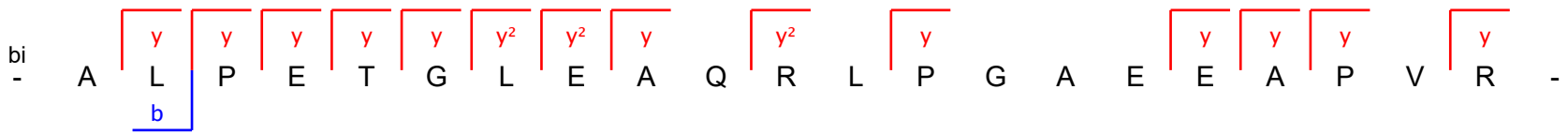
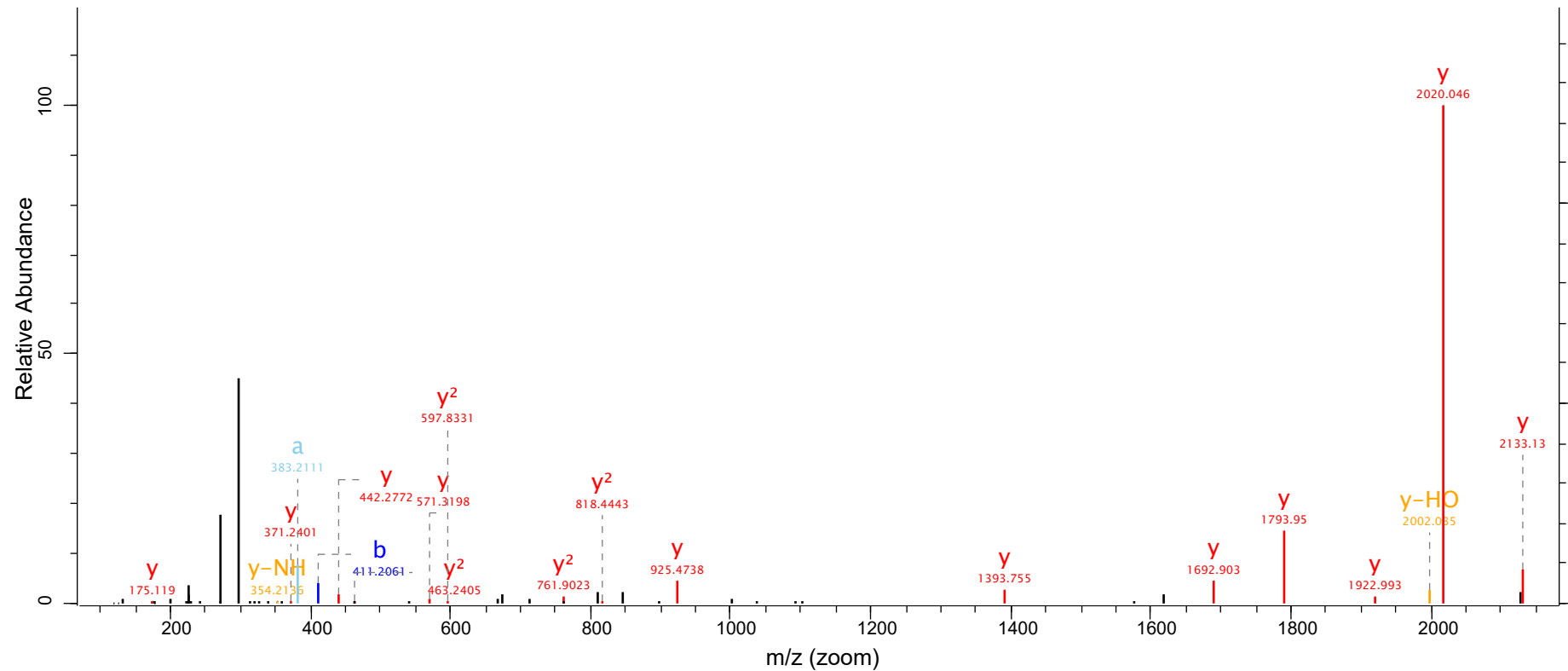
Scan
20986

Method
FTMS; HCD

Score
78.67

m/z
811.09

Gene names
KIF7



Raw File
agg_pep_sort_pos_2

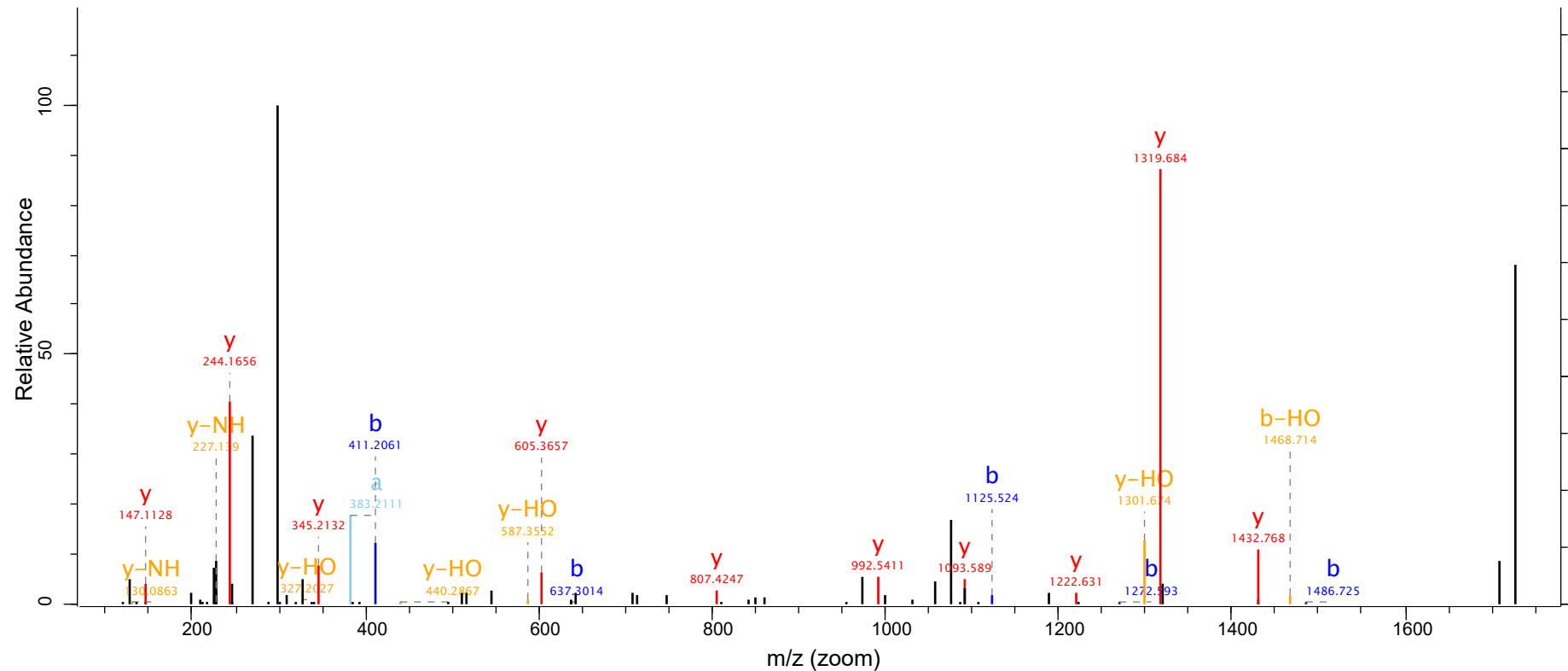
Scan
19811

Method
FTMS; HCD

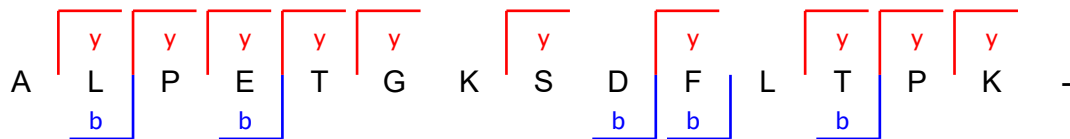
Score
117.09

m/z
865.44

Gene names
KIN



bi



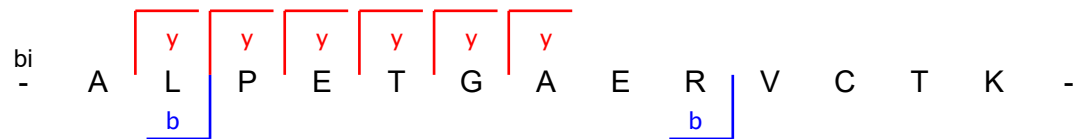
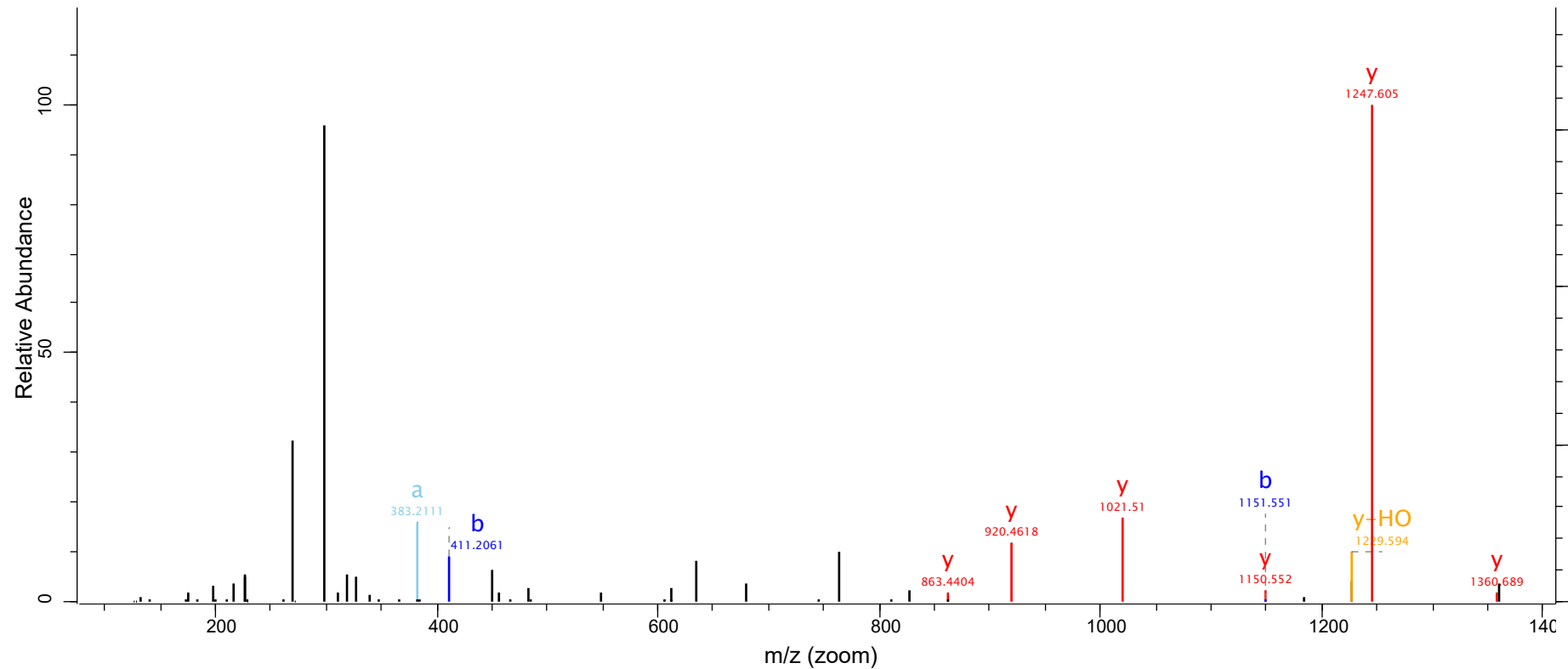
Raw File
agg_pep_sort_pos_3

Scan
23228

Method
FTMS; HCD

Score
61.35

m/z
829.91



Raw File
agg_pep_sort_pos_1

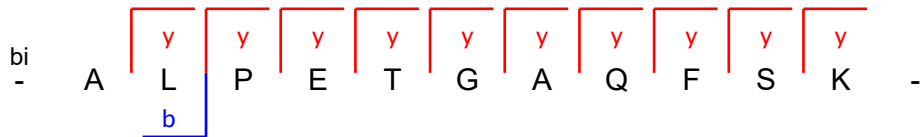
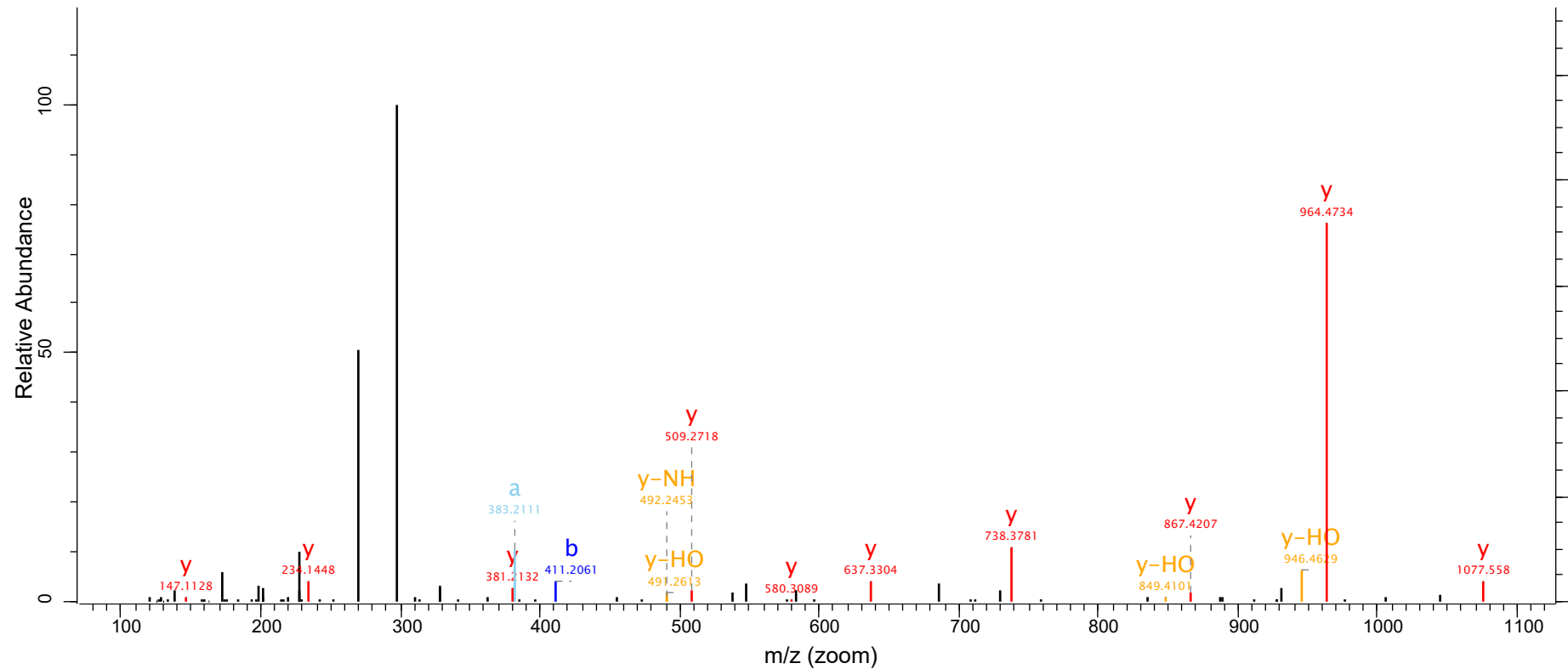
Scan
20808

Method
FTMS; HCD

Score
98.05

m/z
687.84

Gene names
MARCKS



Raw File
agg_pep_sort_pos_3

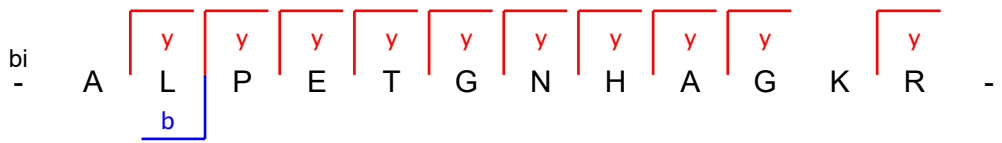
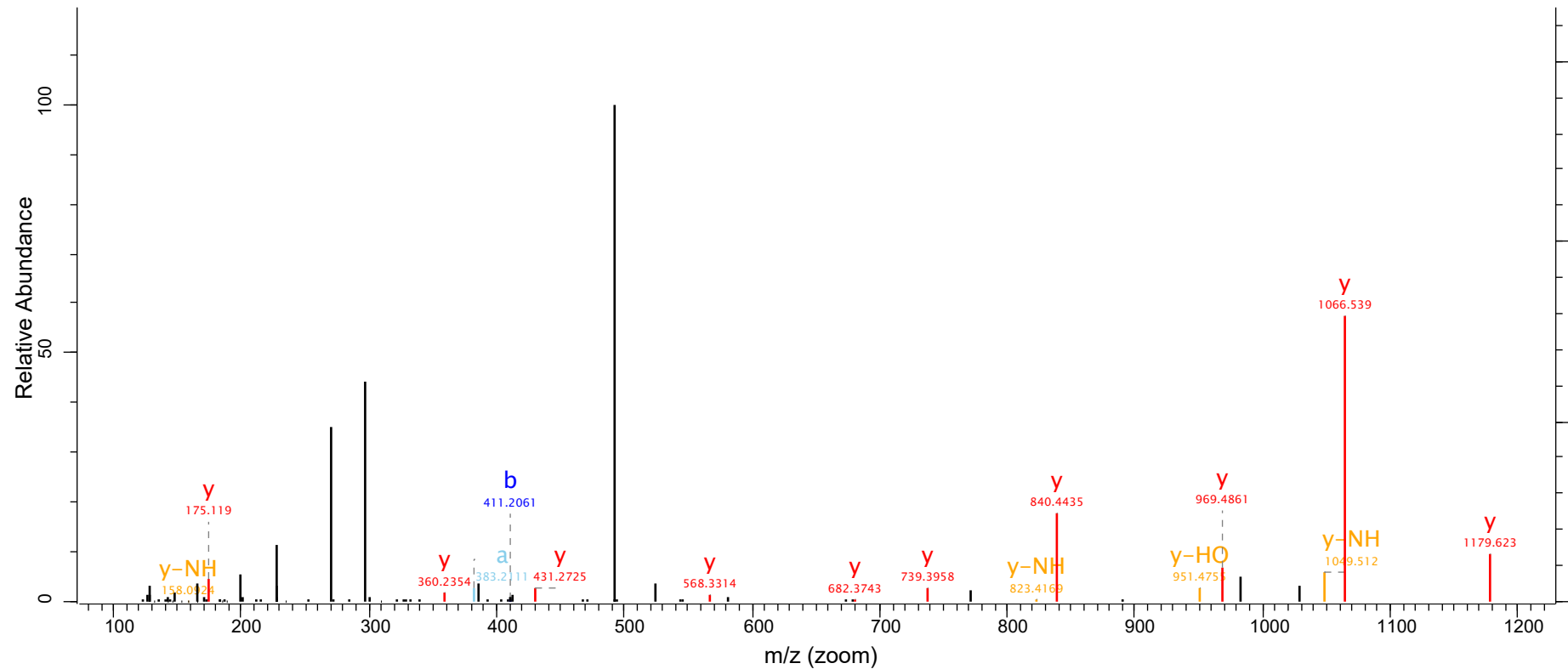
Scan
9068

Method
FTMS; HCD

Score
89.4

m/z
492.92

Gene names
MBP



Raw File
agg_pep_sort_pos_3

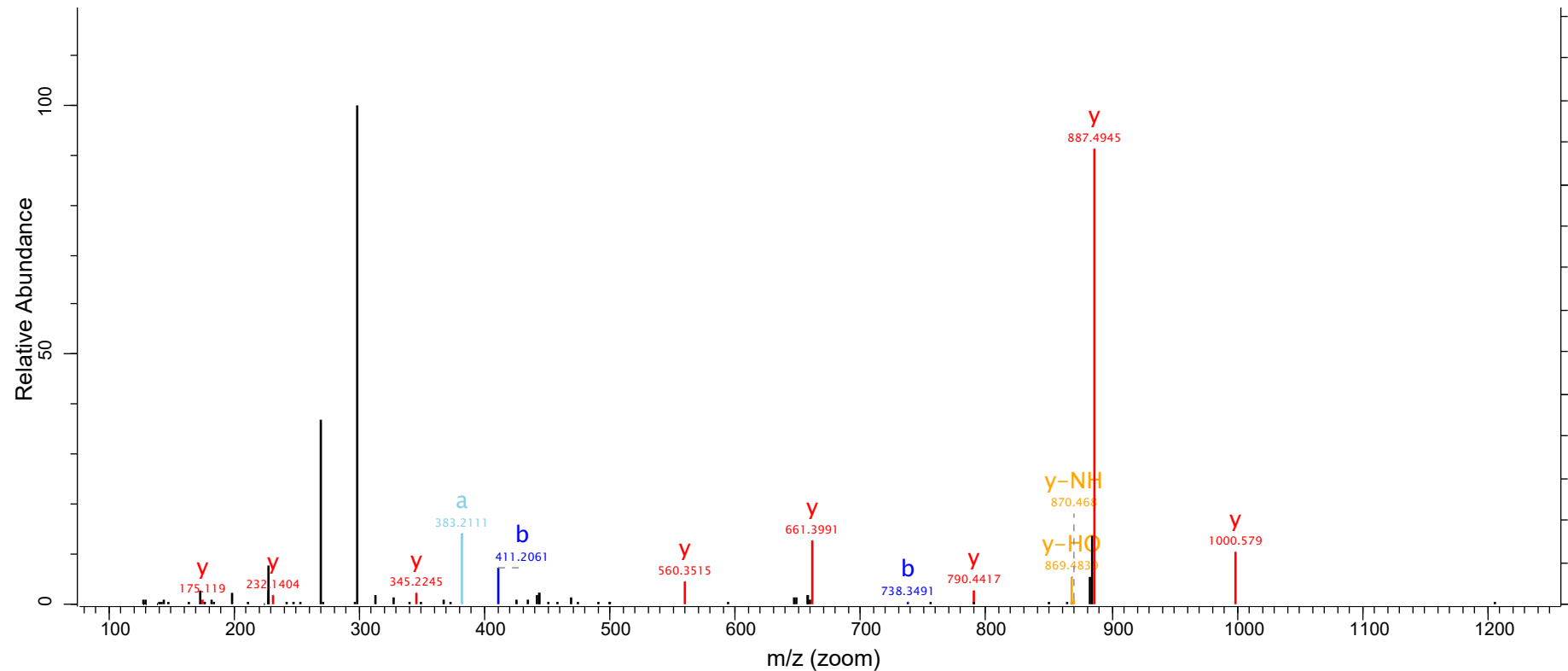
Scan
15847

Method
FTMS; HCD

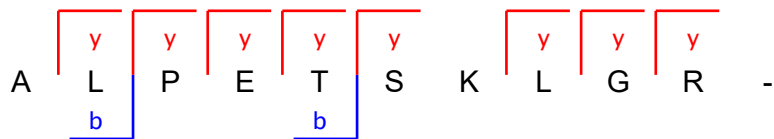
Score
78.15

m/z
649.35

Gene names
MRPL11



bi



Raw File
agg_pep_sort_pos_2

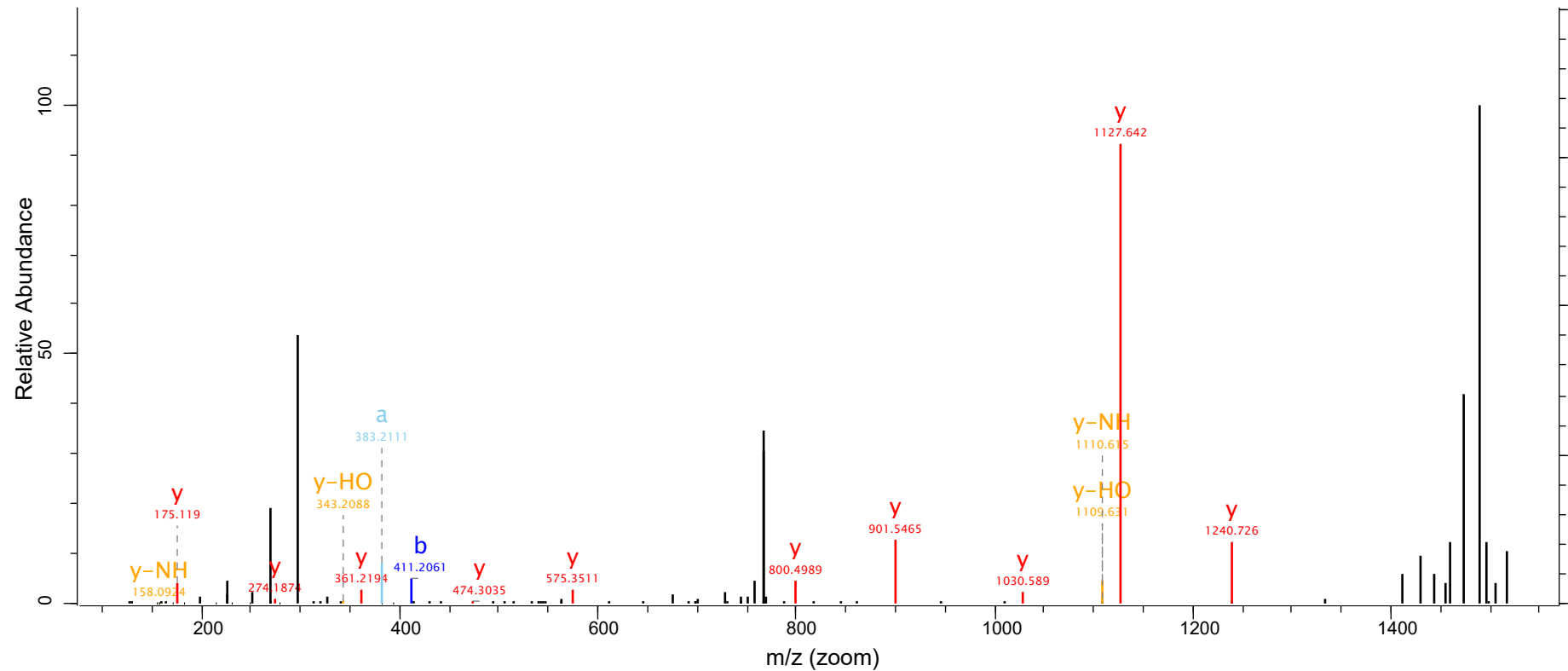
Scan
17309

Method
FTMS; HCD

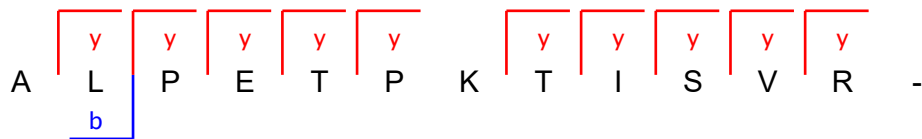
Score
88.68

m/z
769.42

Gene names
MSN



bi



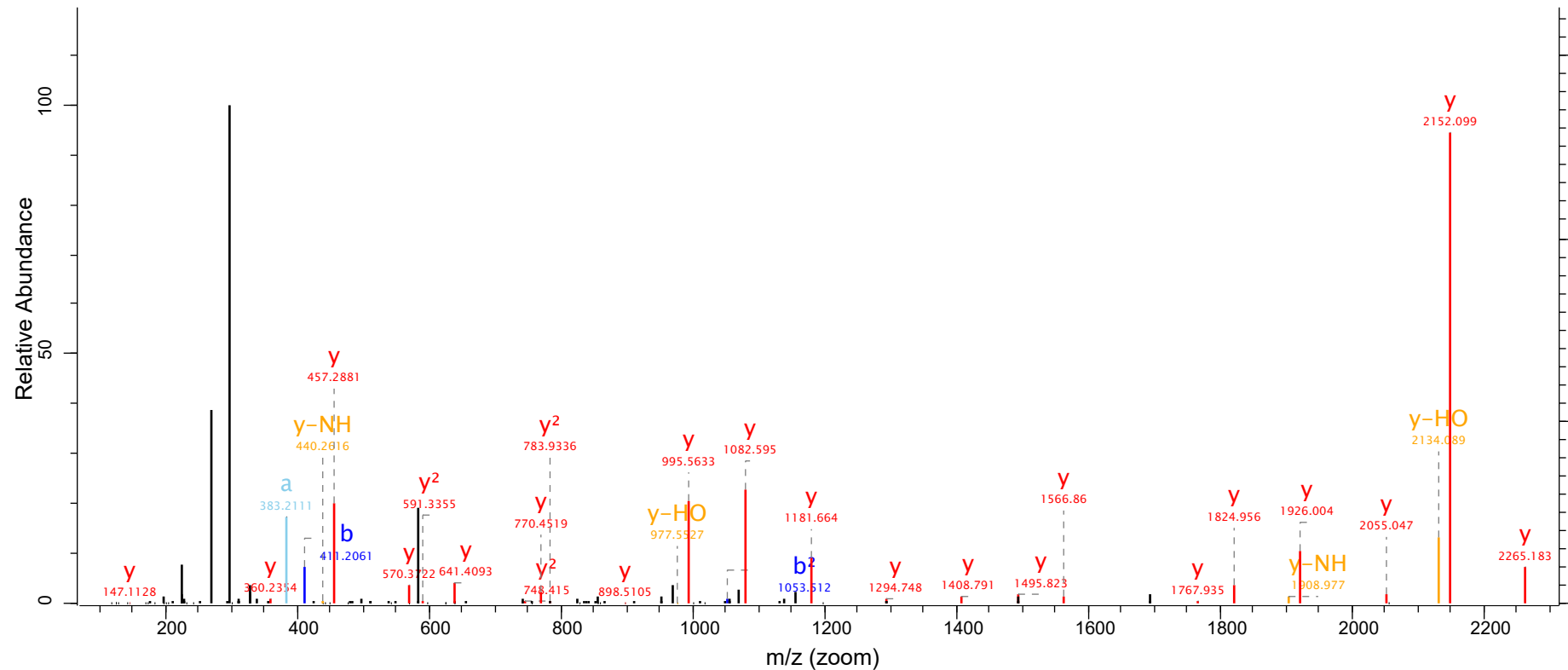
Raw File
agg_pep_sort_pos_3

Scan
19693

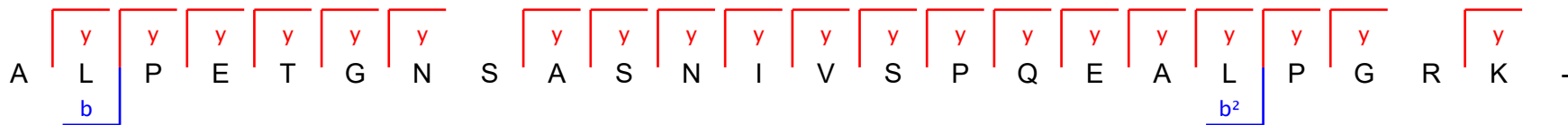
Method
FTMS; HCD

Score
108.4

m/z
855.1



bi



Raw File
agg_pep_sort_pos_1

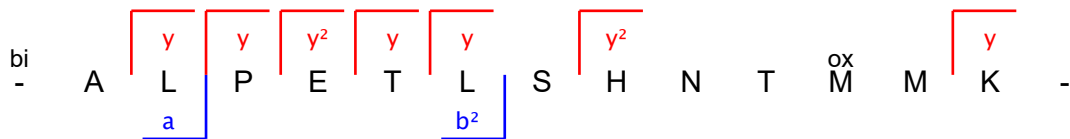
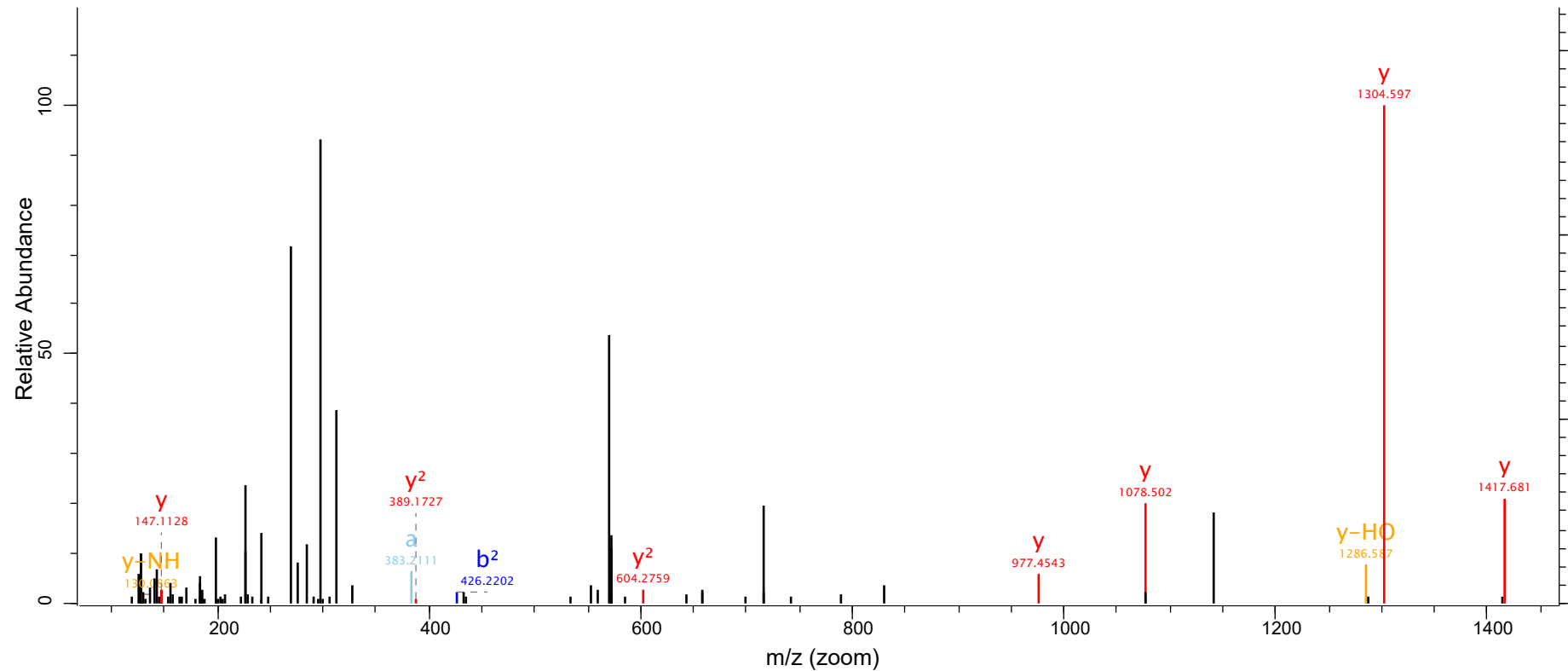
Scan
13552

Method
FTMS; HCD

Score
39.66

m/z
572.27

Gene names
MYO22



Raw File
agg_pep_sort_pos_1

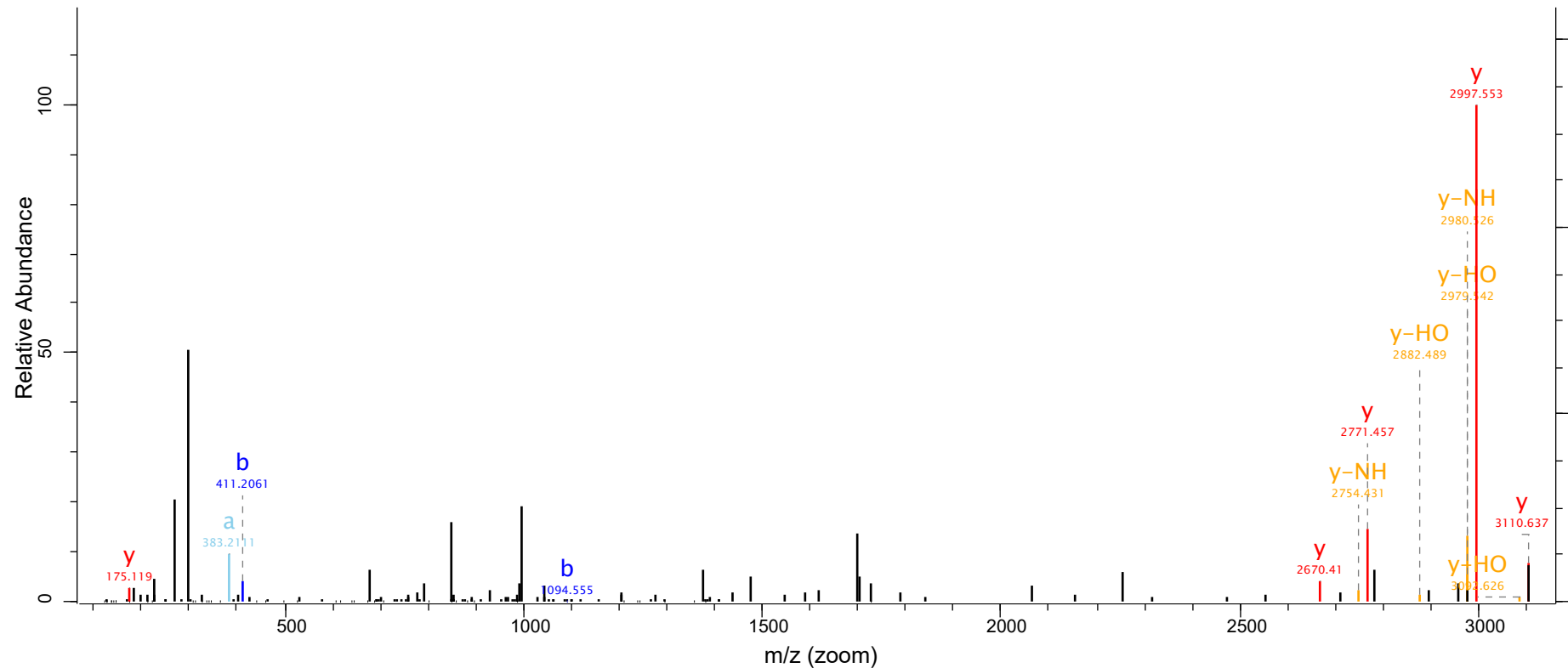
Scan
17626

Method
FTMS; HCD

Score
40.43

m/z
852.94

Gene names
NBPF1



bi - A L P E T V V S A G P W S S E K A E T N I L E I
N E K L R -

Fragmentation mapping:
- Red brackets above the sequence indicate y-ions: L, P, T, V, A, G, P, W, S, S, E, K, A, E, T, N, I, L, E, I.
- Blue brackets below the sequence indicate b-ions: L, P, A, G.

Raw File
agg_pep_sort_pos_2

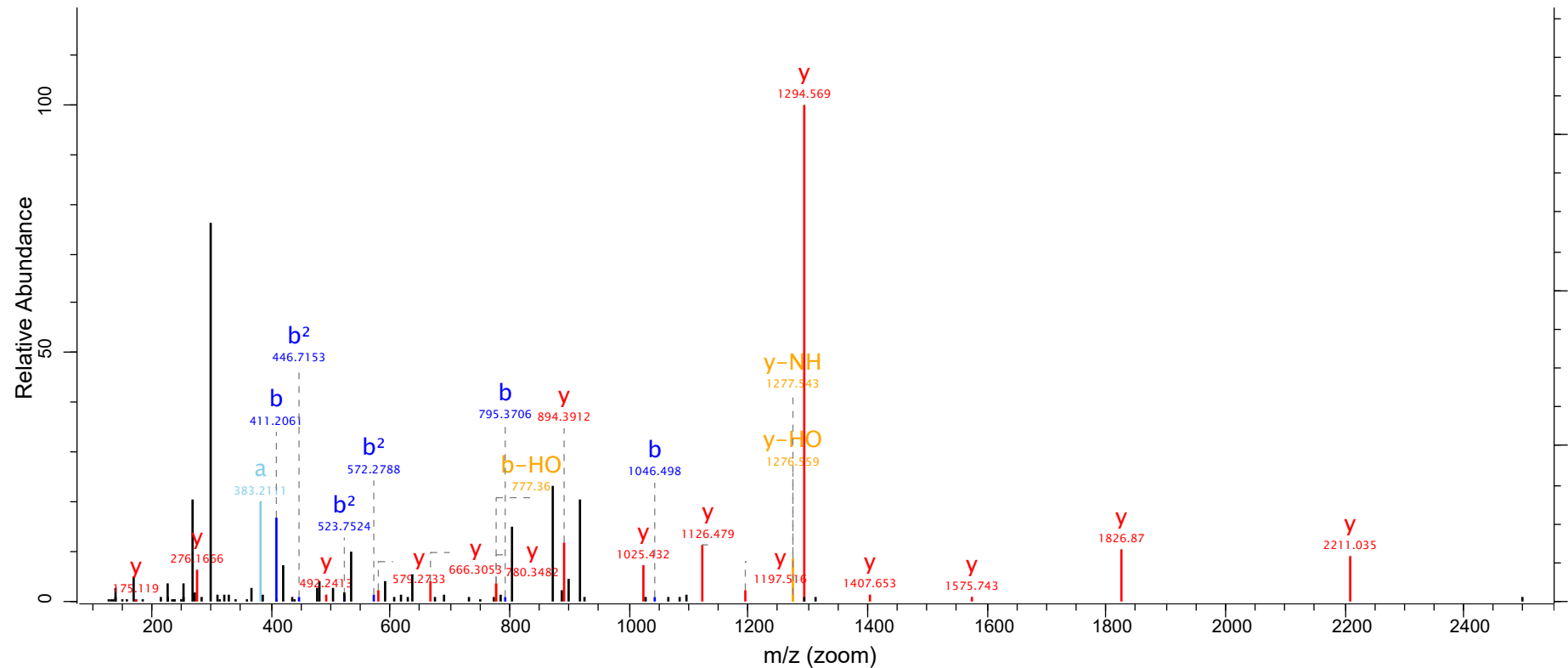
Scan
23621

Method
FTMS; HCD

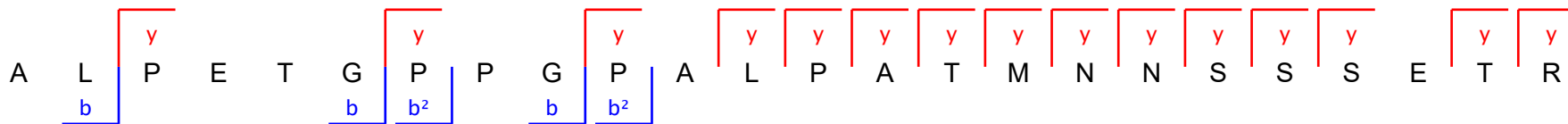
Score
72.8

m/z
874.75

Gene names
NCAPH



bi



Raw File
agg_pep_sort_pos_2

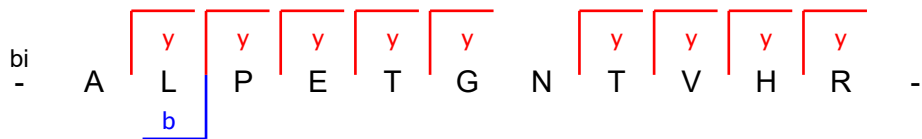
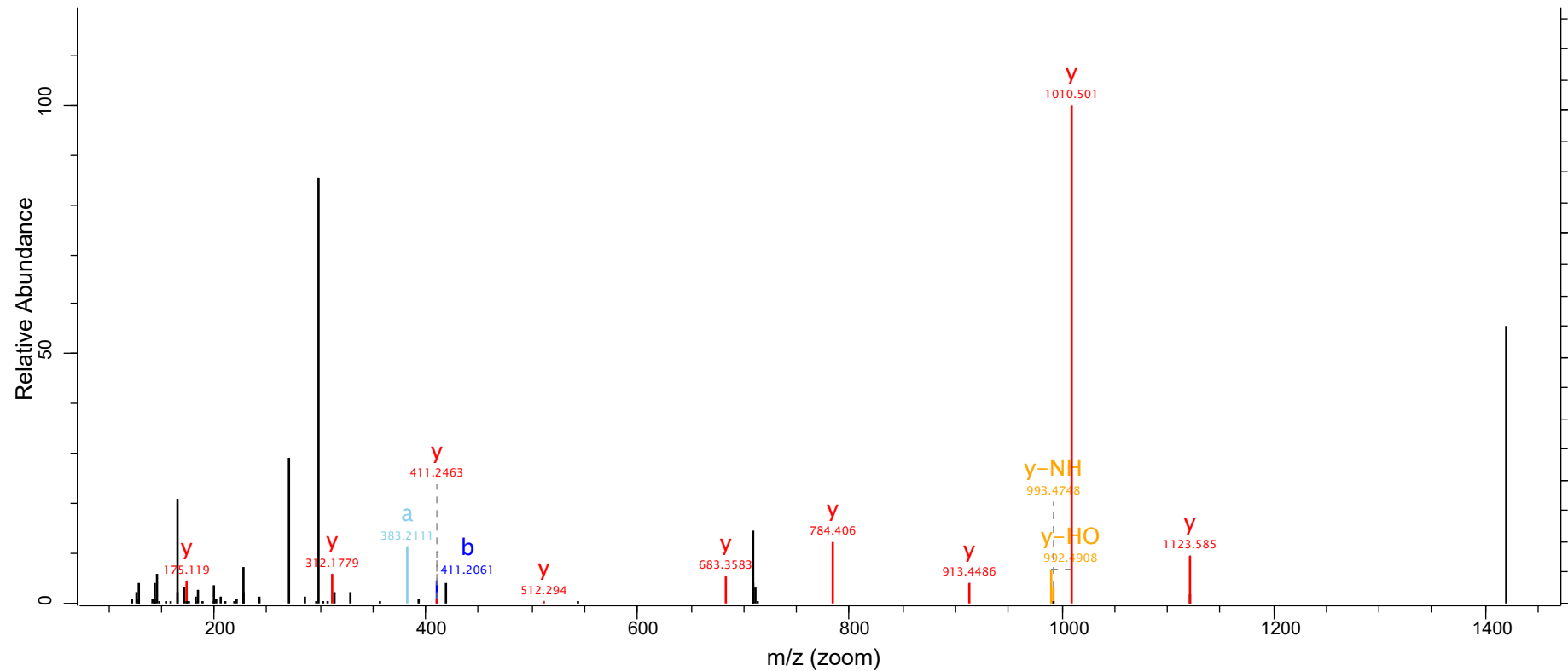
Scan
13981

Method
FTMS; HCD

Score
82.29

m/z
710.85

Gene names
NEURL1B



Raw File
agg_pep_sort_pos_1

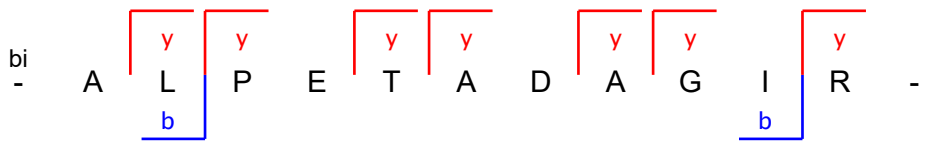
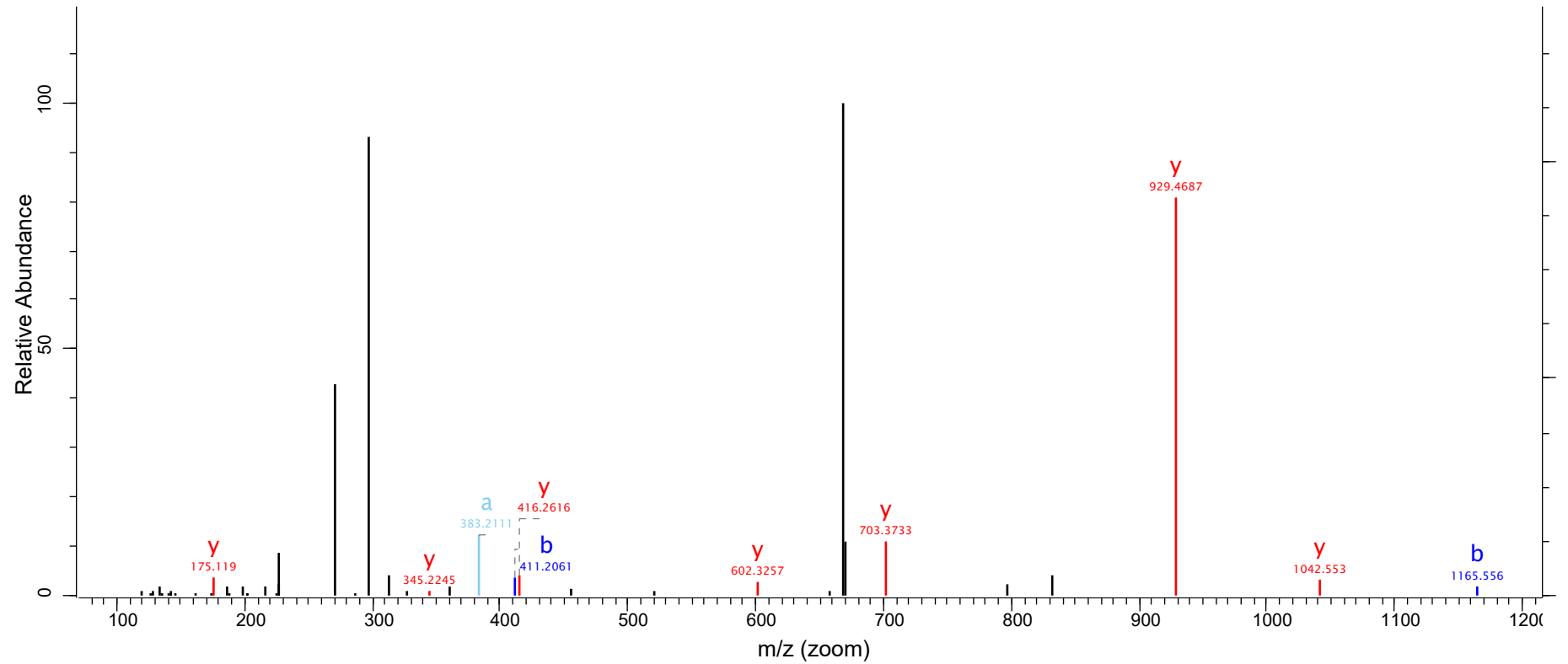
Scan
21547

Method
FTMS; HCD

Score
65.31

m/z
670.34

Gene names
NOLC1



Raw File
agg_pep_sort_pos_1

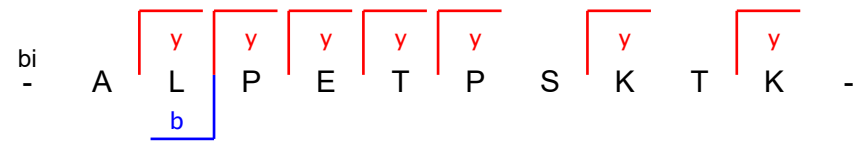
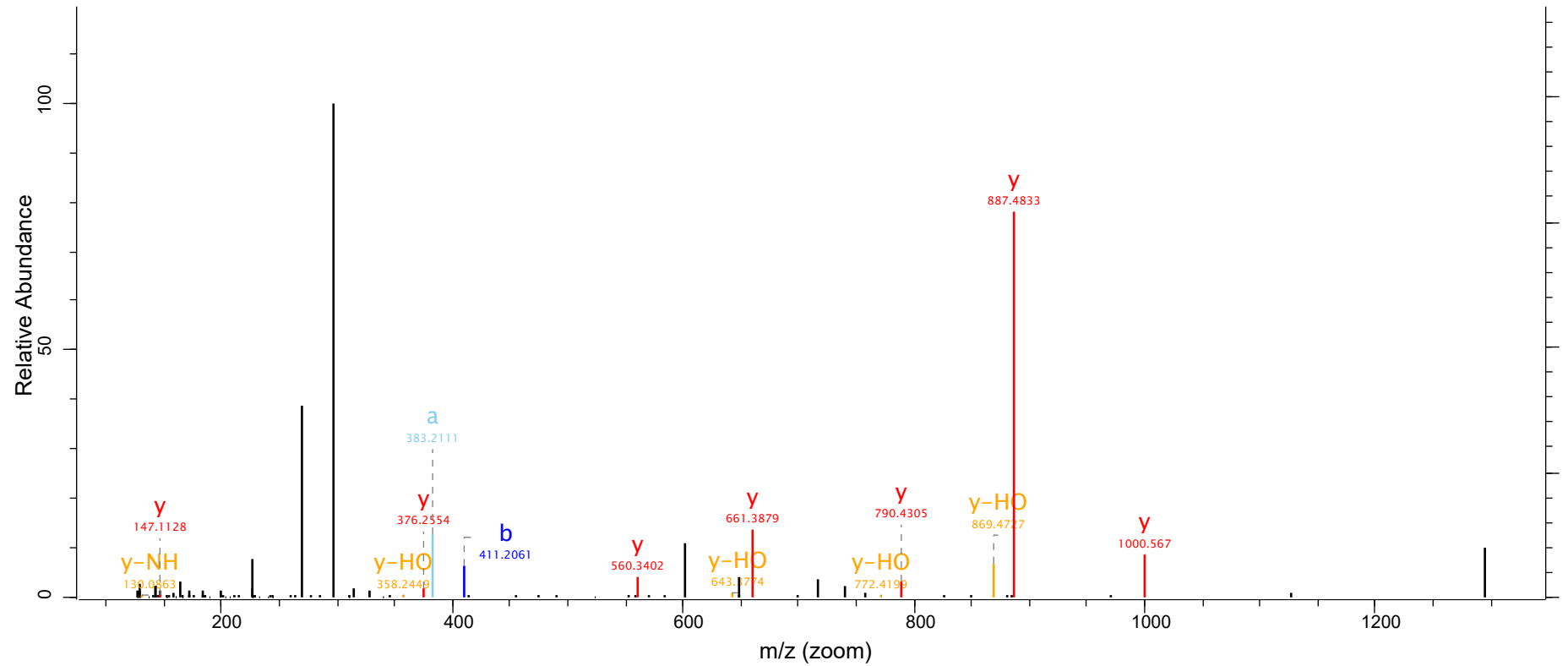
Scan
13372

Method
FTMS; HCD

Score
78.33

m/z
649.34

Gene names
NOS1AP



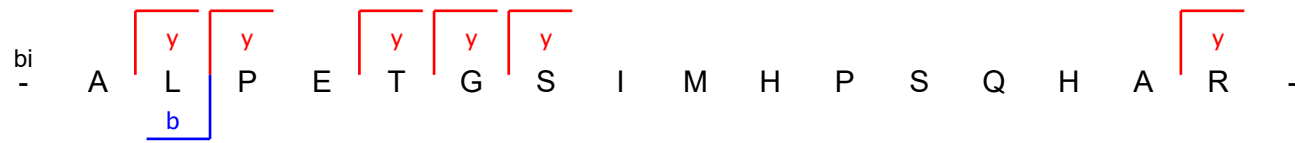
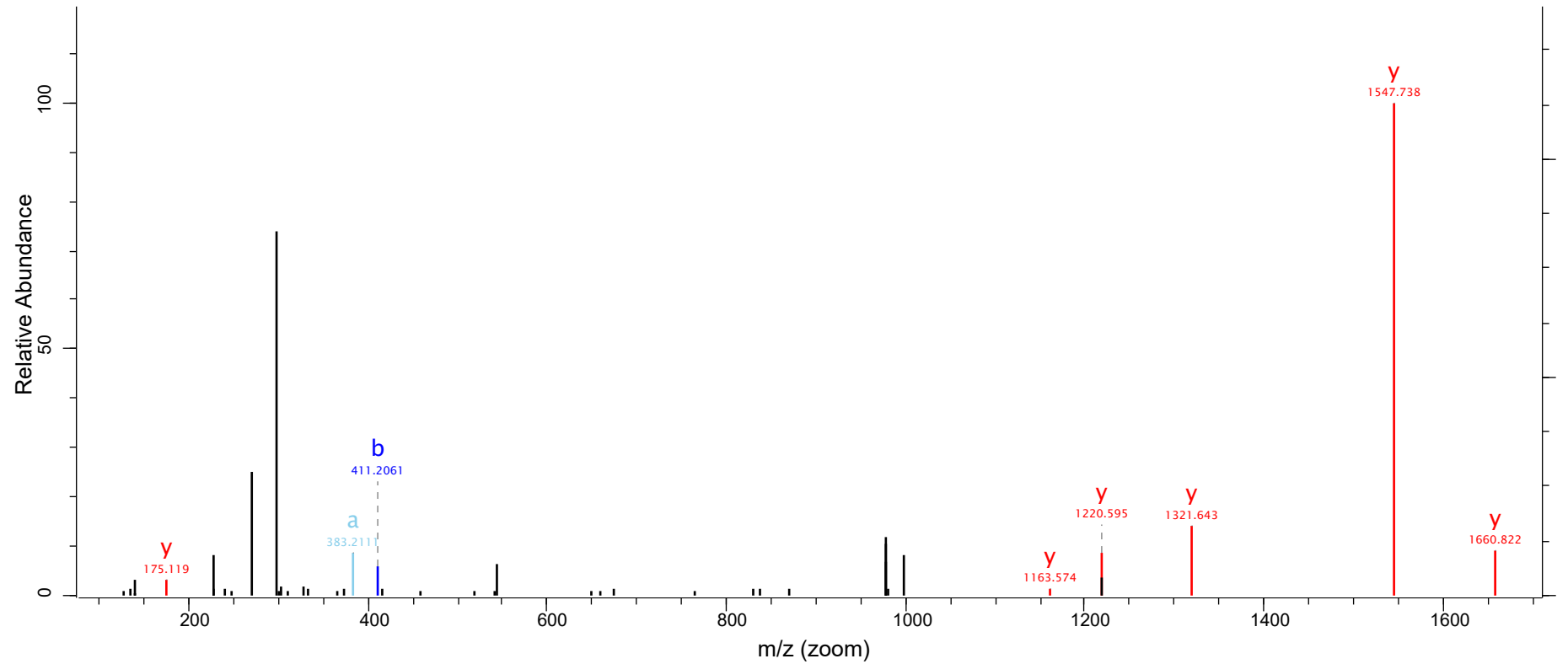
Raw File
agg_pep_sort_pos_1

Scan
20894

Method
FTMS; HCD

Score
47.29

m/z
979.97



Raw File
agg_pep_sort_pos_2

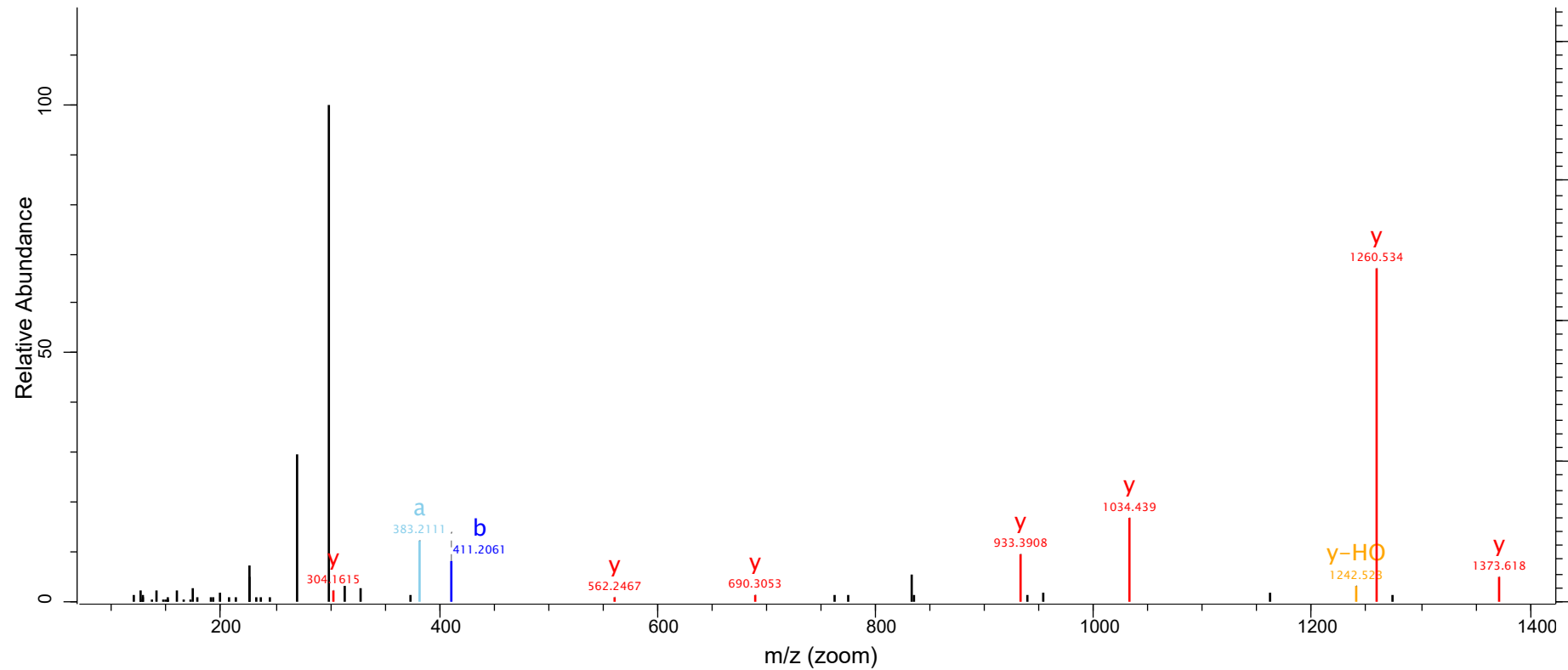
Scan
17596

Method
FTMS; HCD

Score
61.35

m/z
835.87

Gene names
NUDC



bi



Raw File
agg_pep_sort_pos_2

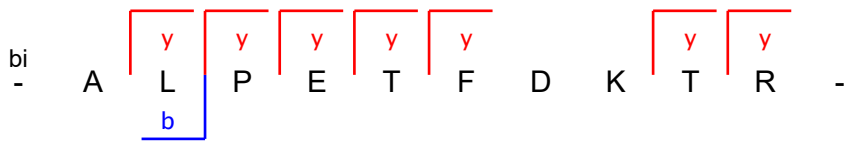
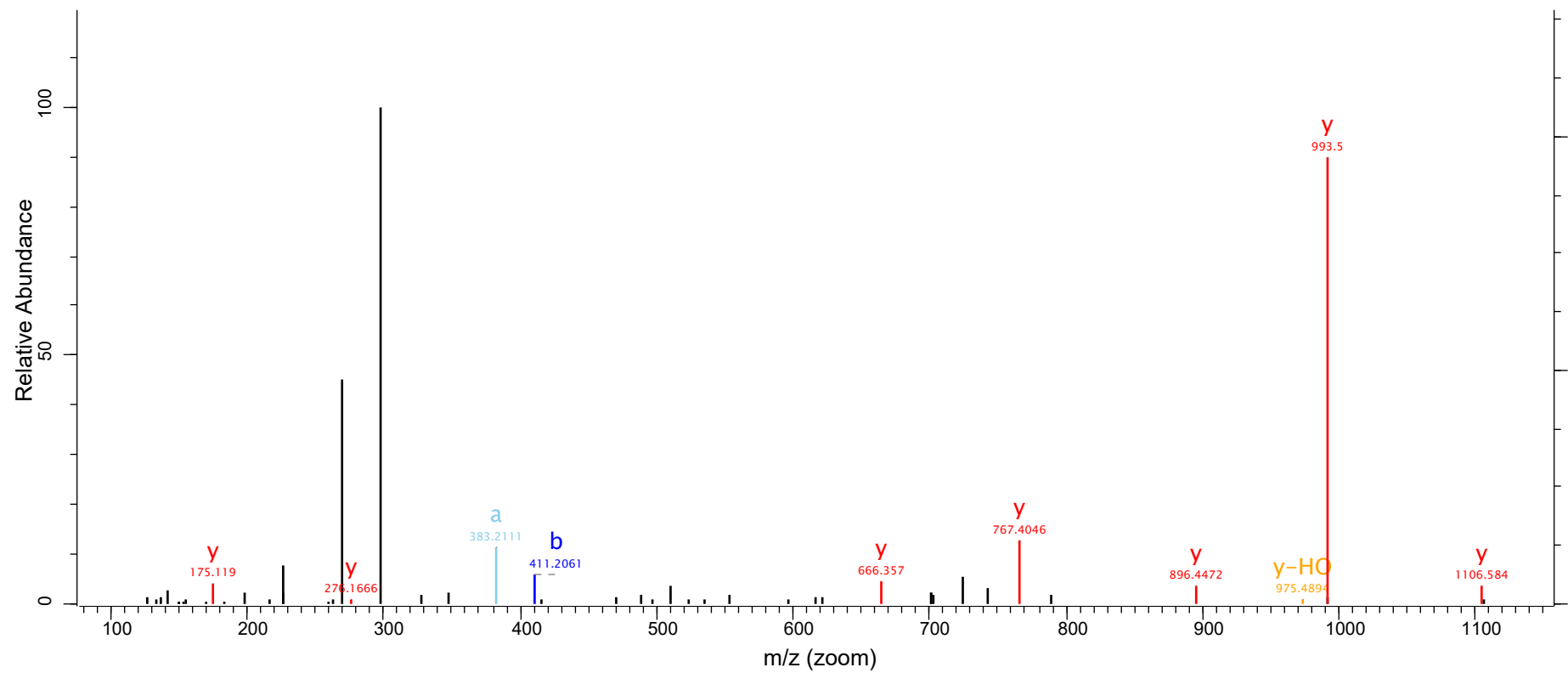
Scan
21201

Method
FTMS; HCD

Score
68.73

m/z
702.35

Gene names
PPAP2A



Raw File
agg_pep_sort_pos_1

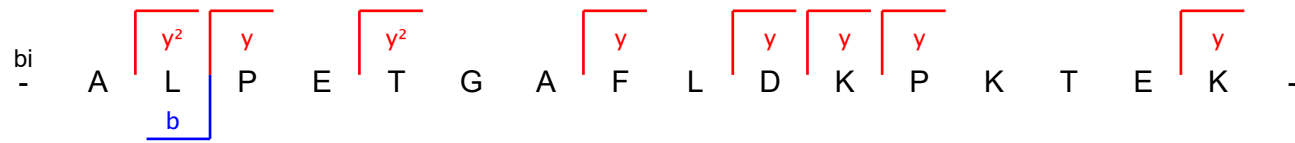
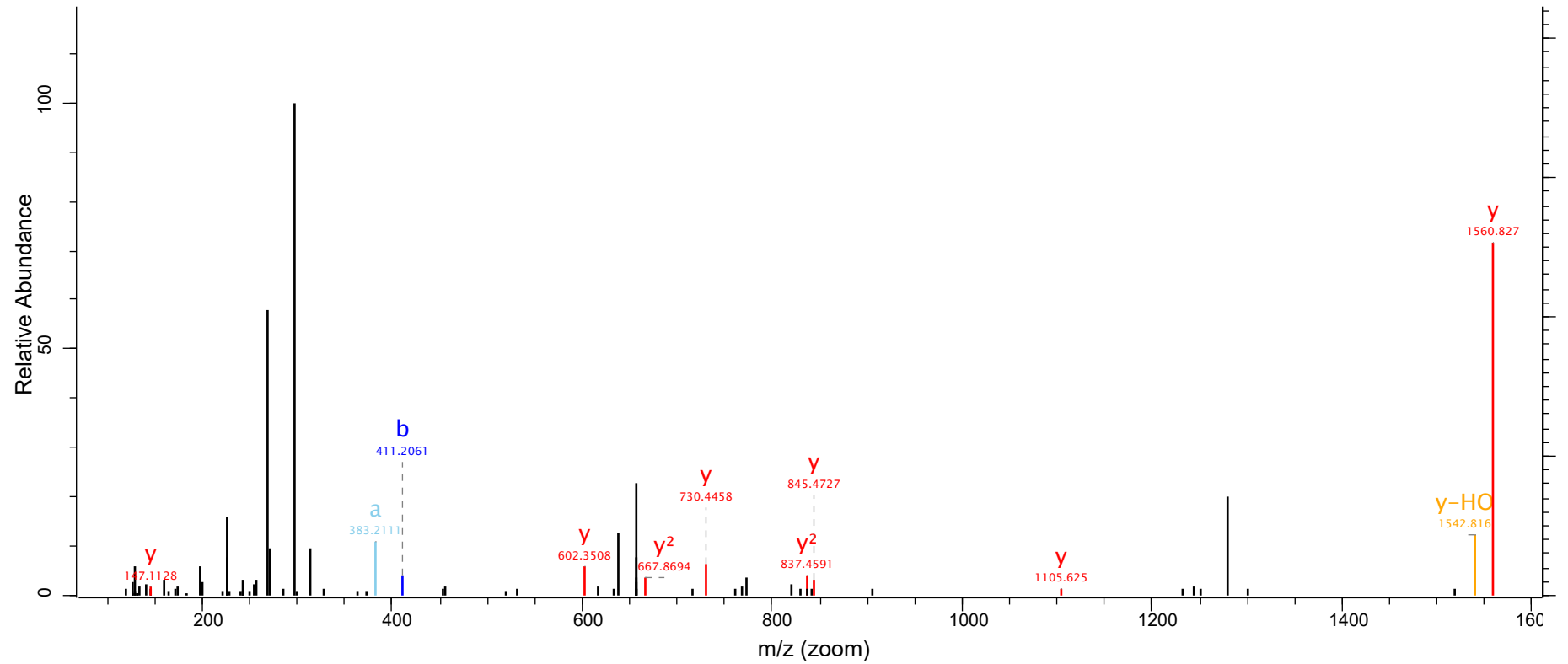
Scan
17696

Method
FTMS; HCD

Score
45.33

m/z
658.01

Gene names
PPM1B



Raw File
agg_pep_sort_pos_1

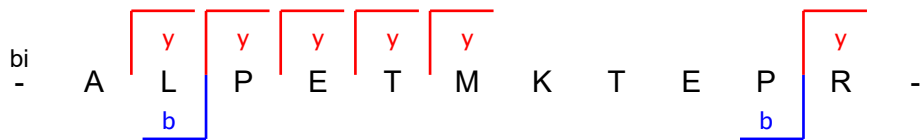
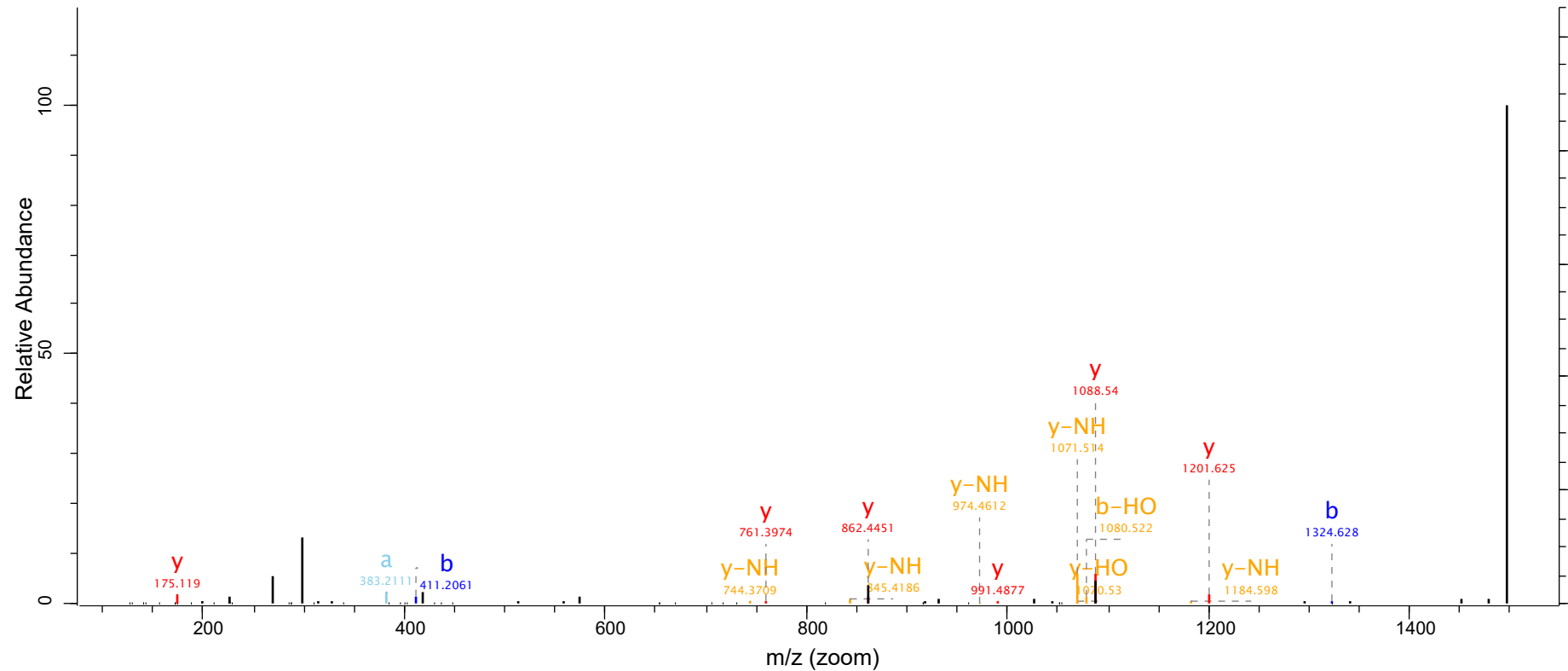
Scan
17865

Method
FTMS; HCD

Score
112.84

m/z
749.88

Gene names
PPP1R9B



Raw File
agg_pep_sort_pos_3

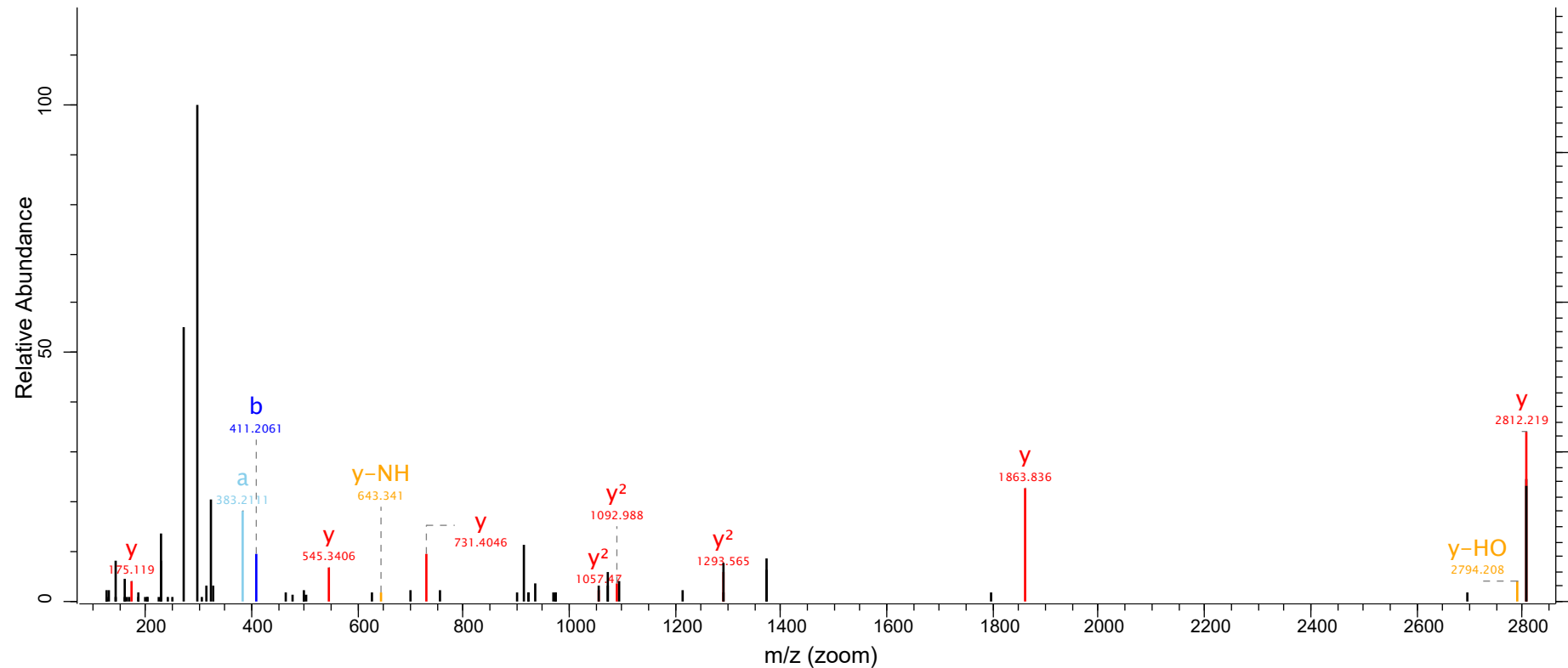
Scan
19856

Method
FTMS; HCD

Score
32.95

m/z
1075.48

Gene names
PPP3R1



bi - A L P E T G N E A S Y P L E M^{ox} C S H F D A D E I
K R -

Fragmentation labels (y, y², b) are shown above the corresponding amino acids in the sequence.

Raw File
agg_pep_sort_pos_3

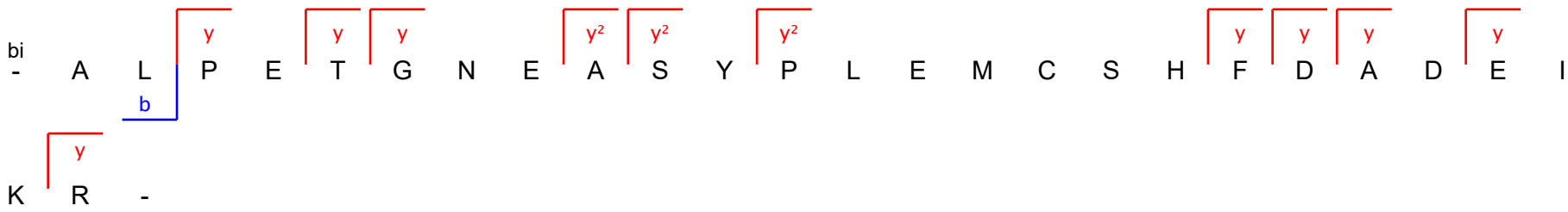
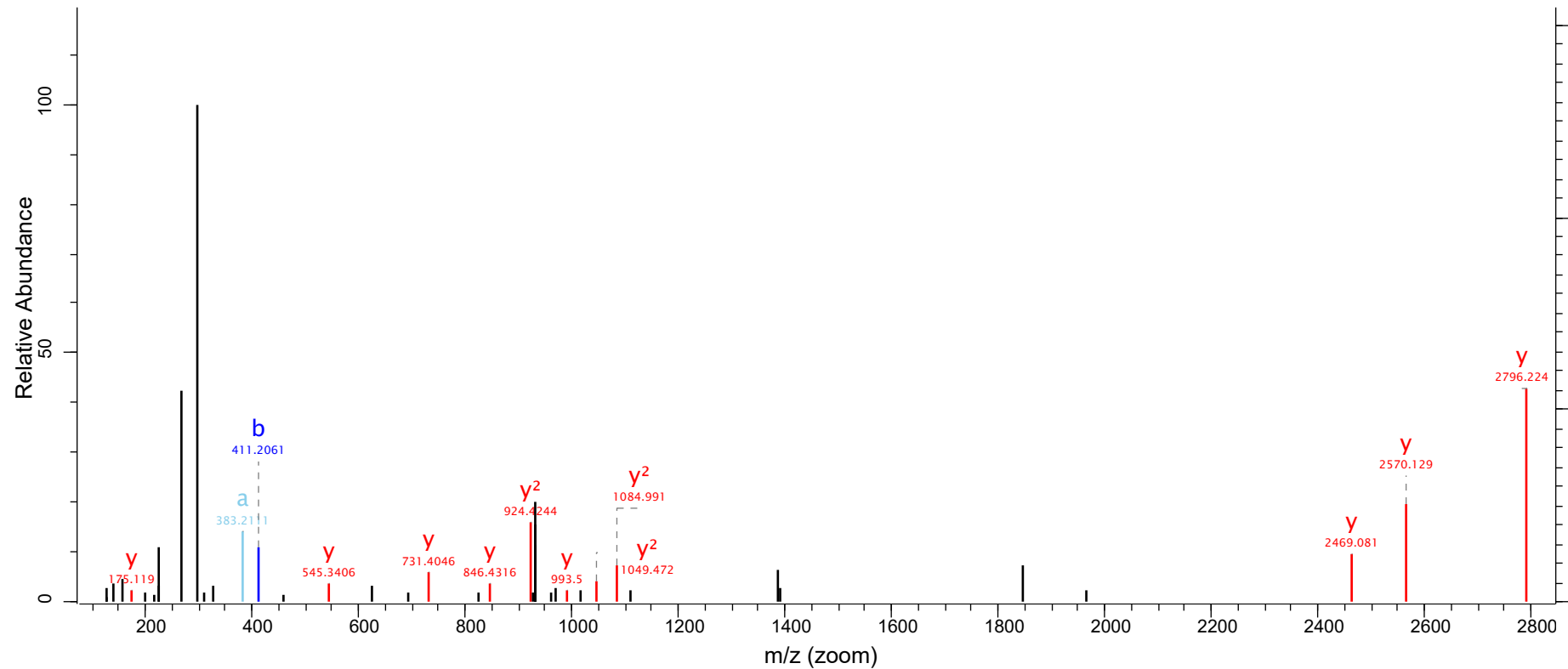
Scan
21538

Method
FTMS; HCD

Score
42.81

m/z
1069.81

Gene names
PPP3R1



Raw File
agg_pep_sort_pos_1

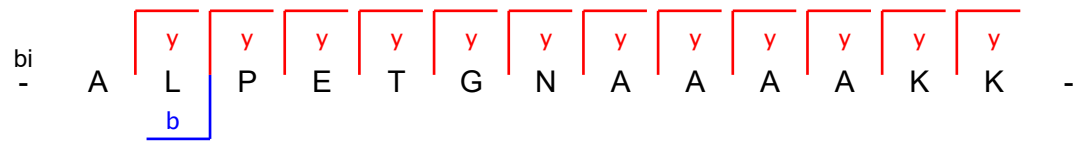
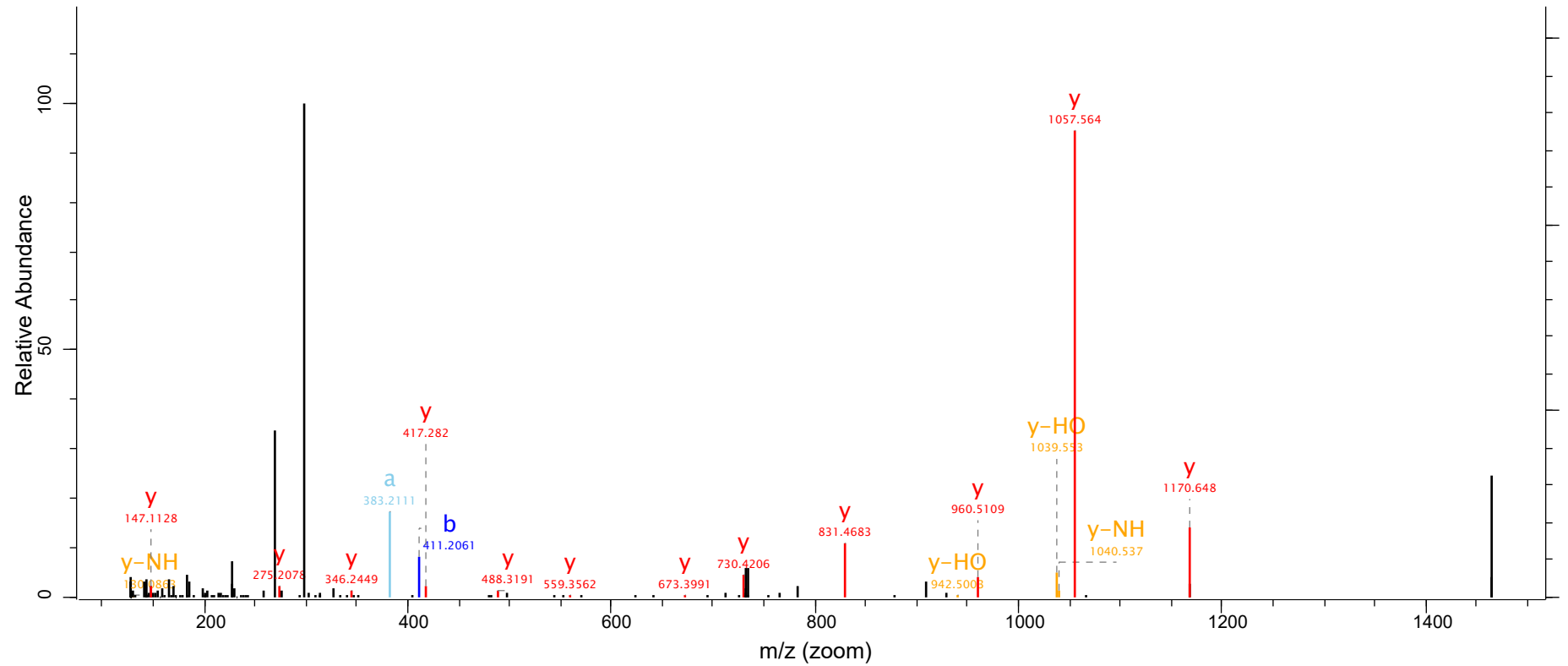
Scan
12752

Method
FTMS; HCD

Score
105.2

m/z
734.39

Gene names
PRKACA



Raw File
agg_pep_sort_pos_1

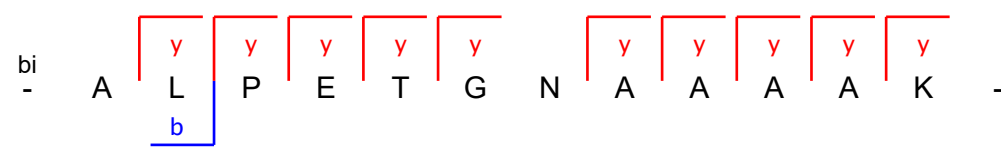
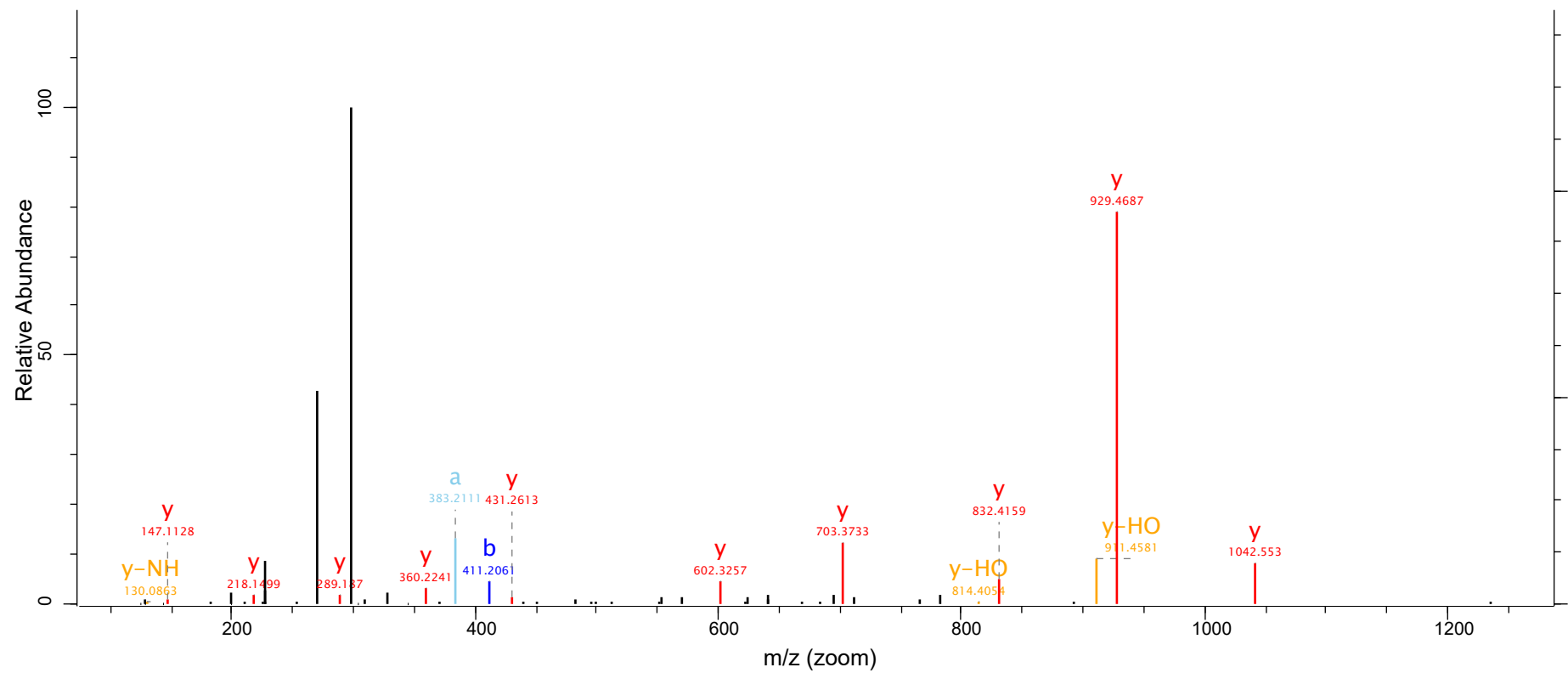
Scan
17256

Method
FTMS; HCD

Score
107.15

m/z
670.34

Gene names
PRKACA



Raw File
agg_pep_sort_pos_1

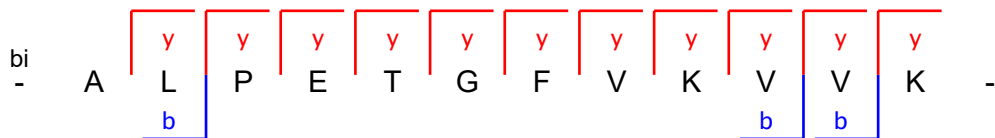
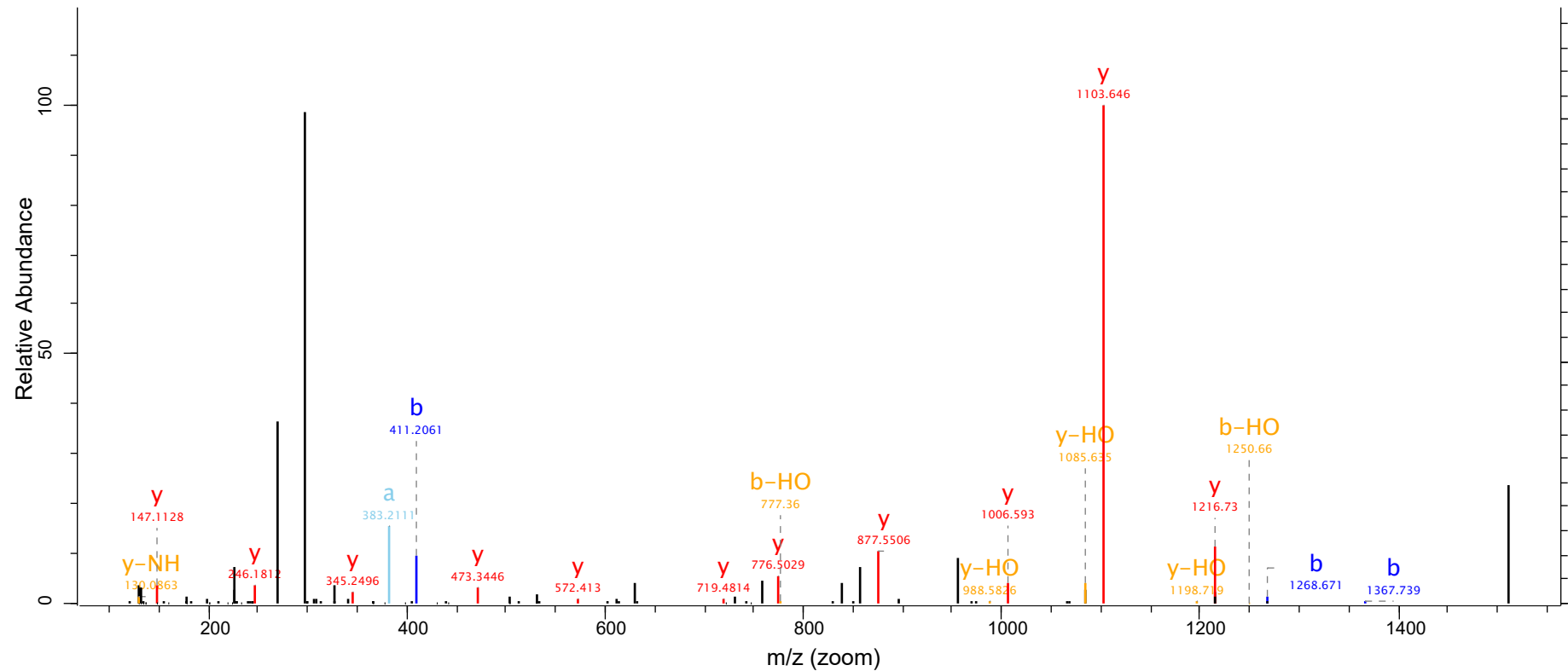
Scan
20947

Method
FTMS; HCD

Score
142.43

m/z
757.43

Gene names
RPL5



Raw File
agg_pep_sort_pos_3

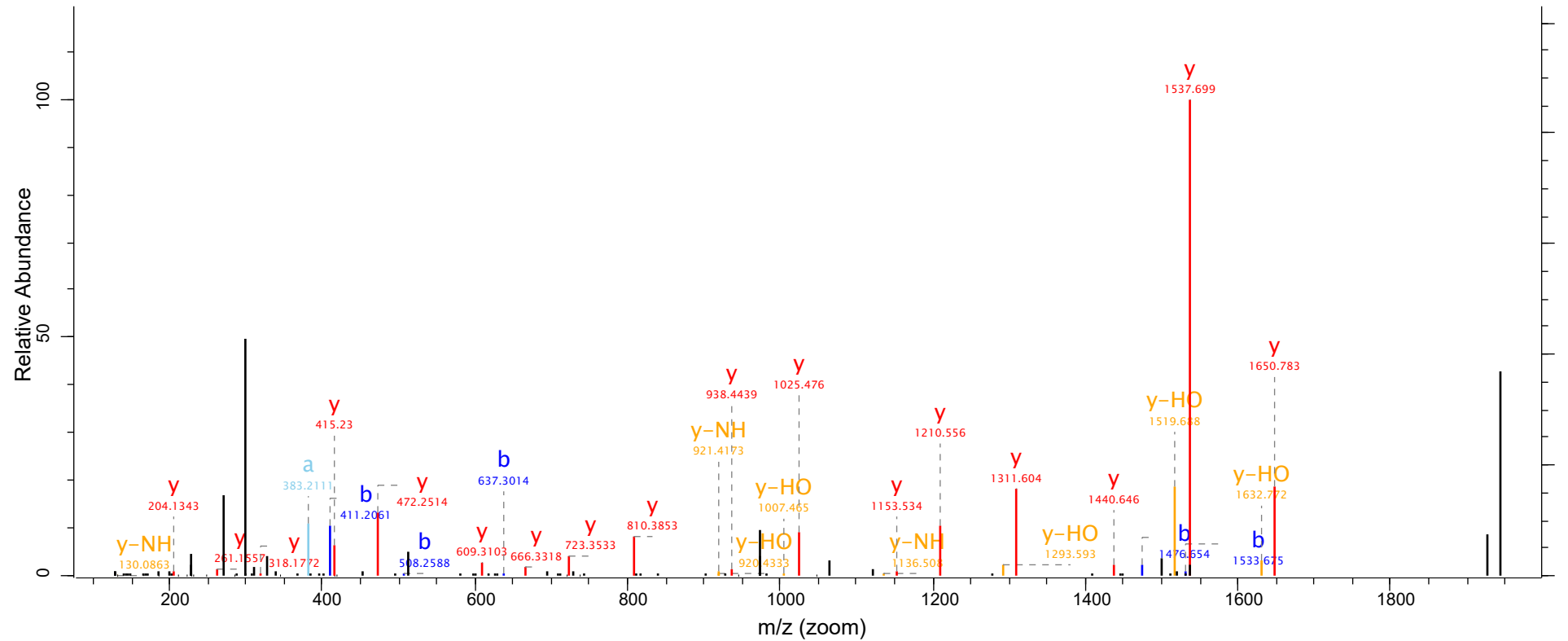
Scan
12249

Method
FTMS; HCD

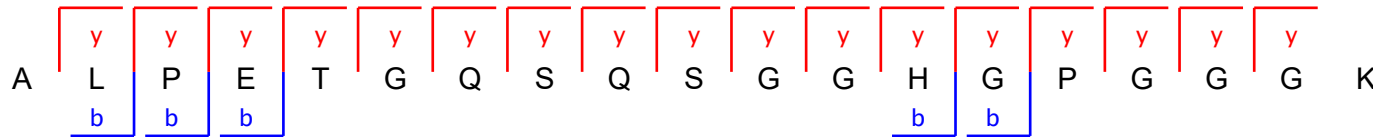
Score
174.39

m/z
974.45

Gene names
PSMC1



bi



Raw File
agg_pep_sort_pos_1

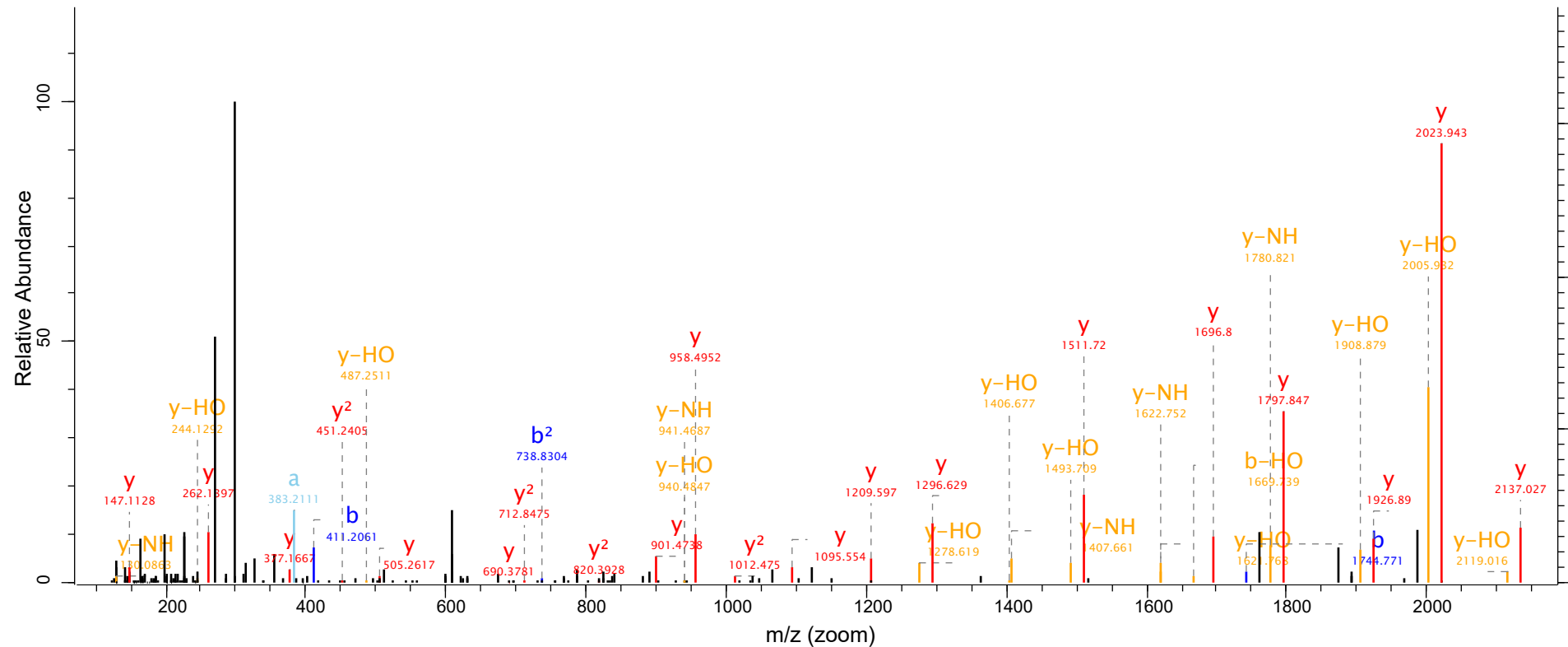
Scan
8569

Method
FTMS; HCD

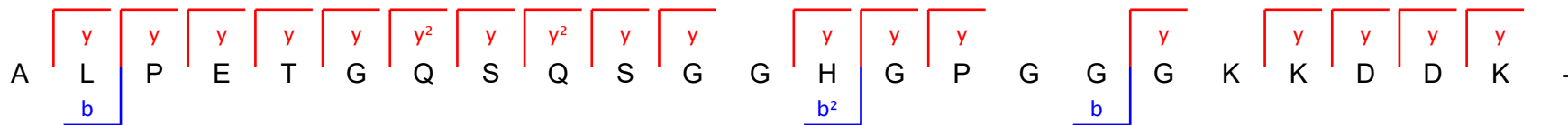
Score
117.38

m/z
609.54

Gene names
PSMC1



bi



Raw File
agg_pep_sort_pos_2

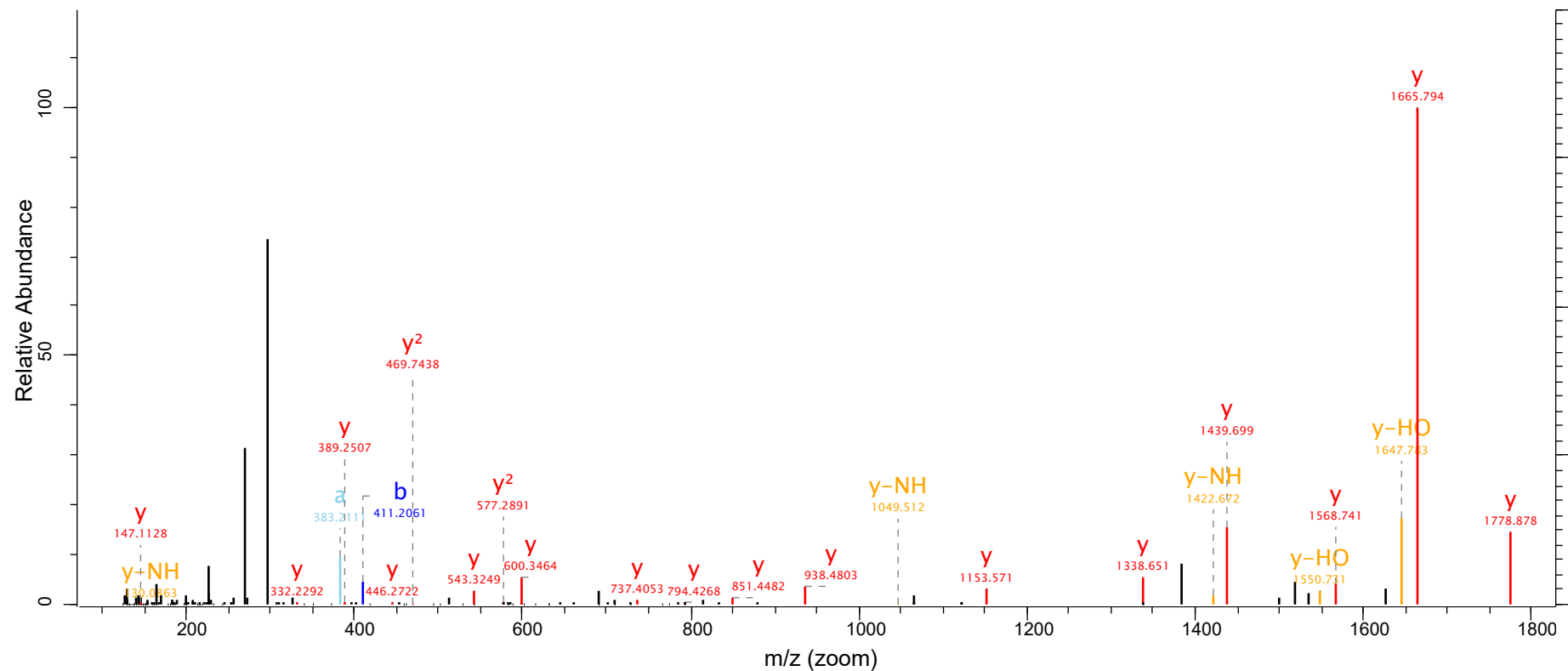
Scan
9365

Method
FTMS; HCD

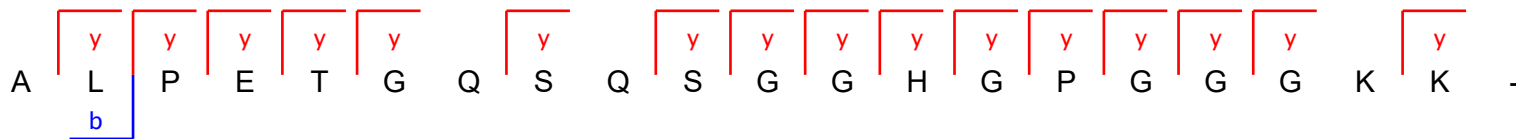
Score
95.21

m/z
693

Gene names
PSMC1



bi



Raw File
agg_pep_sort_pos_3

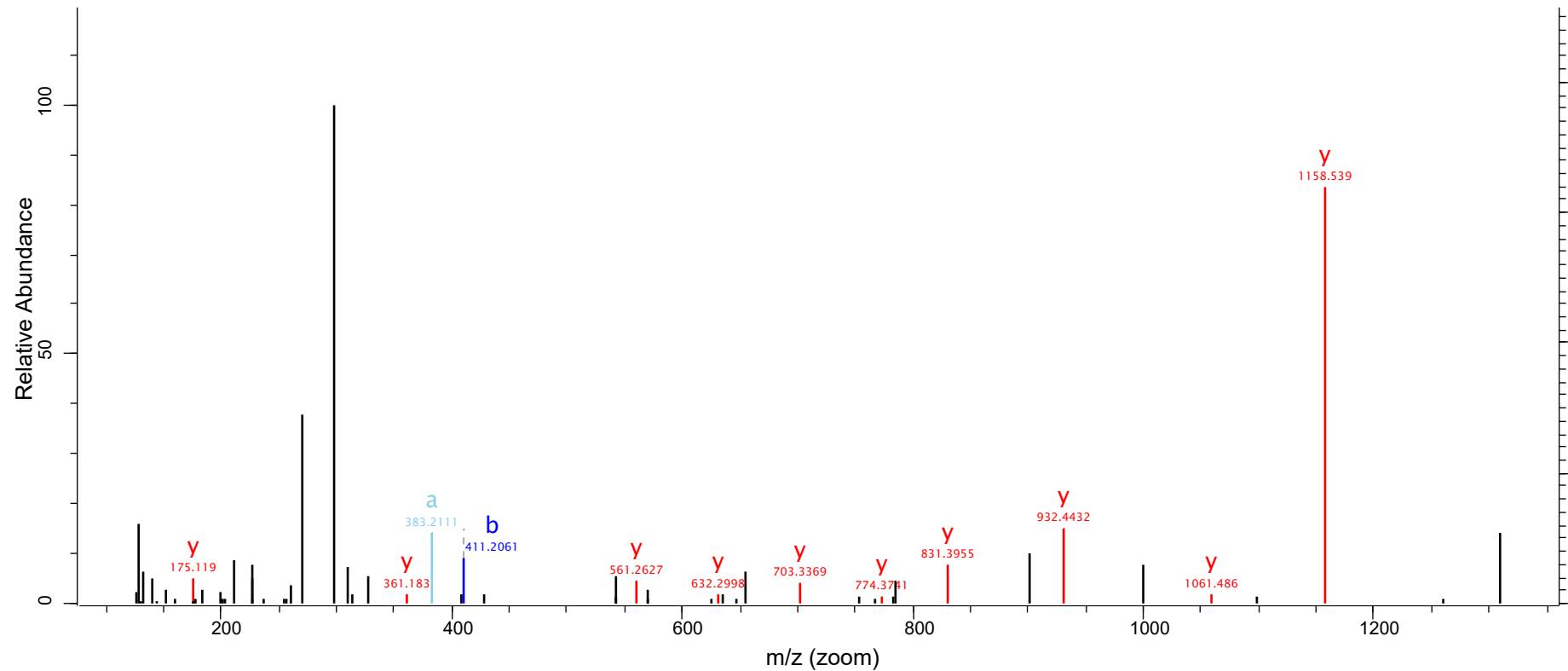
Scan
19264

Method
FTMS; HCD

Score
68.54

m/z
784.87

Gene names
RBM7



bi -
A L P E T G A A A E A D R -
b

Raw File
agg_pep_sort_pos_2

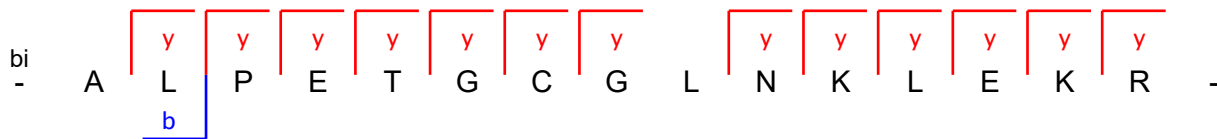
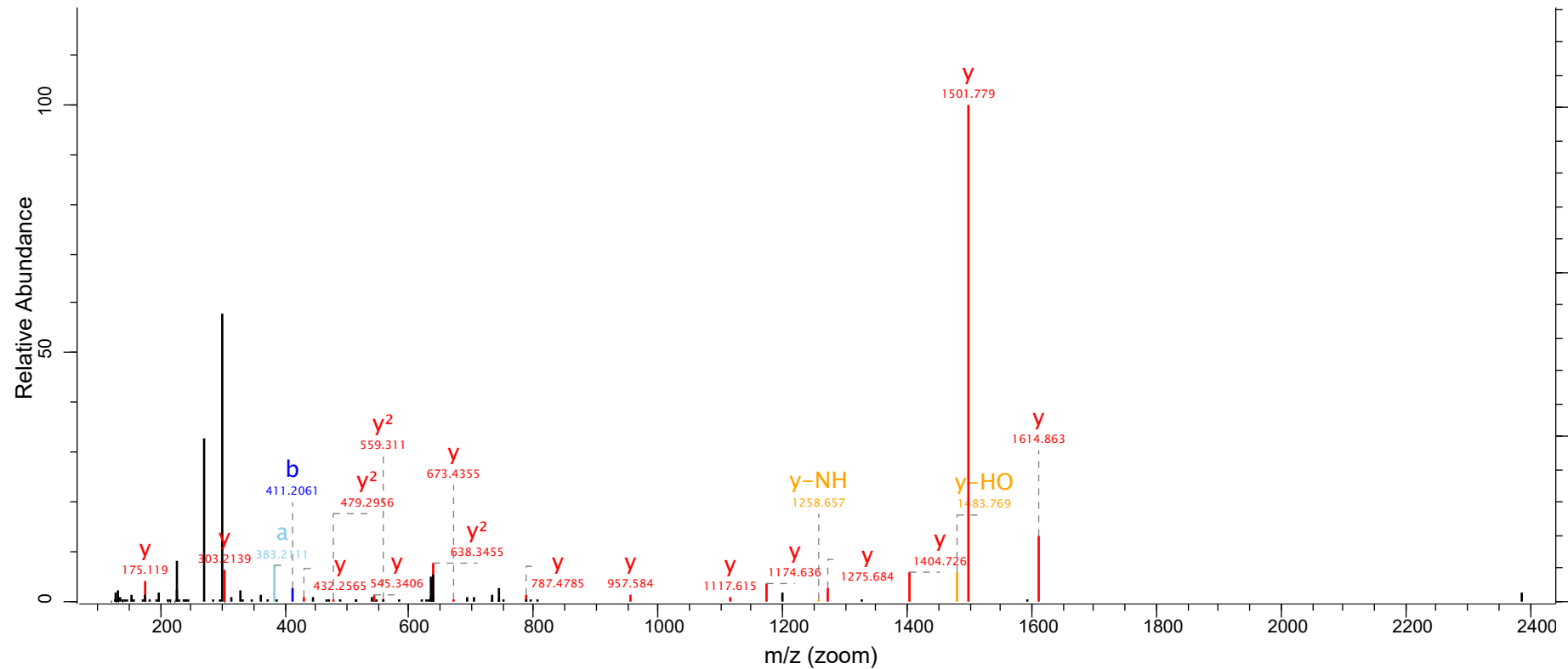
Scan
15298

Method
FTMS; HCD

Score
89.91

m/z
638

Gene names
RFTN1



Raw File
agg_pep_sort_pos_3

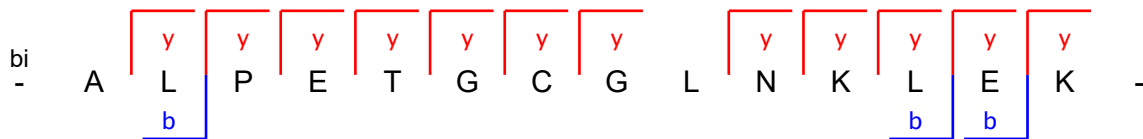
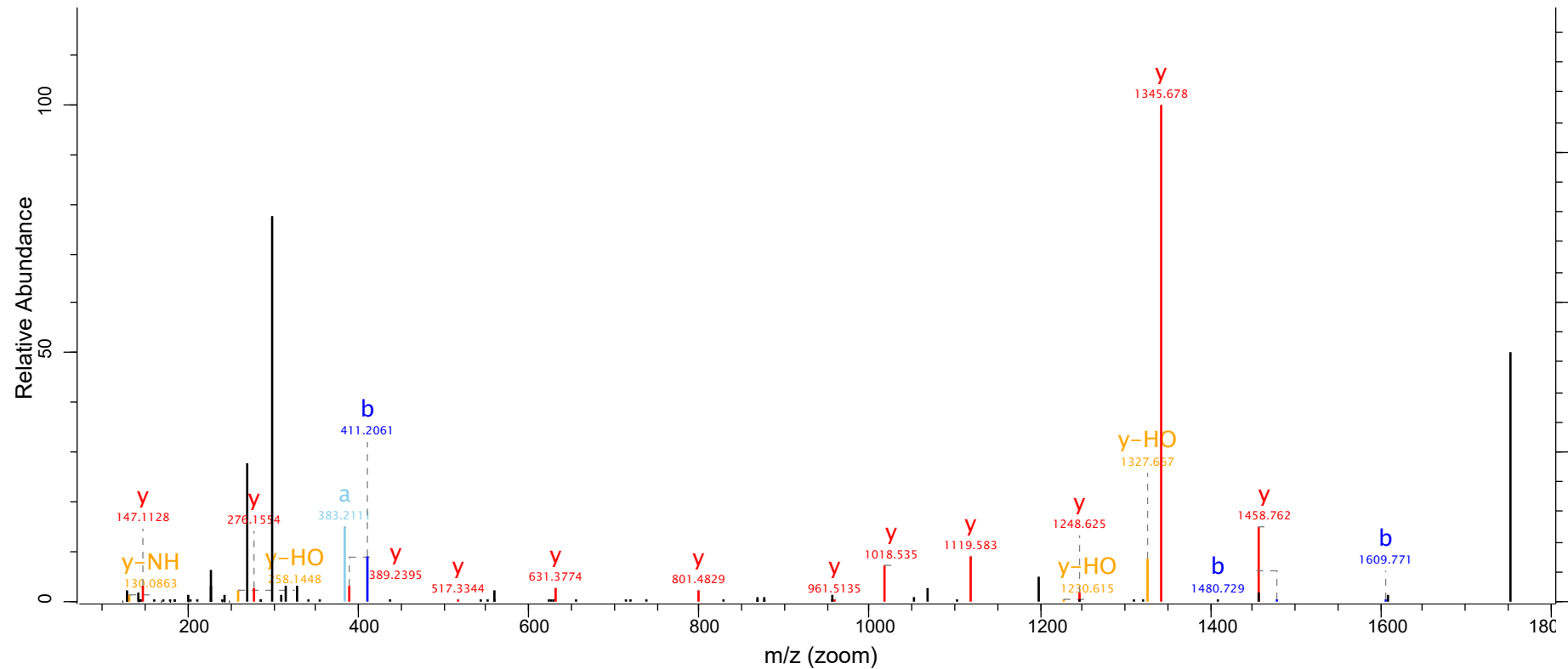
Scan
17788

Method
FTMS; HCD

Score
108.56

m/z
878.44

Gene names
RFTN1



Raw File
agg_pep_sort_pos_3

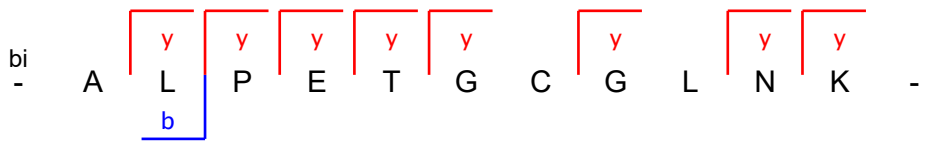
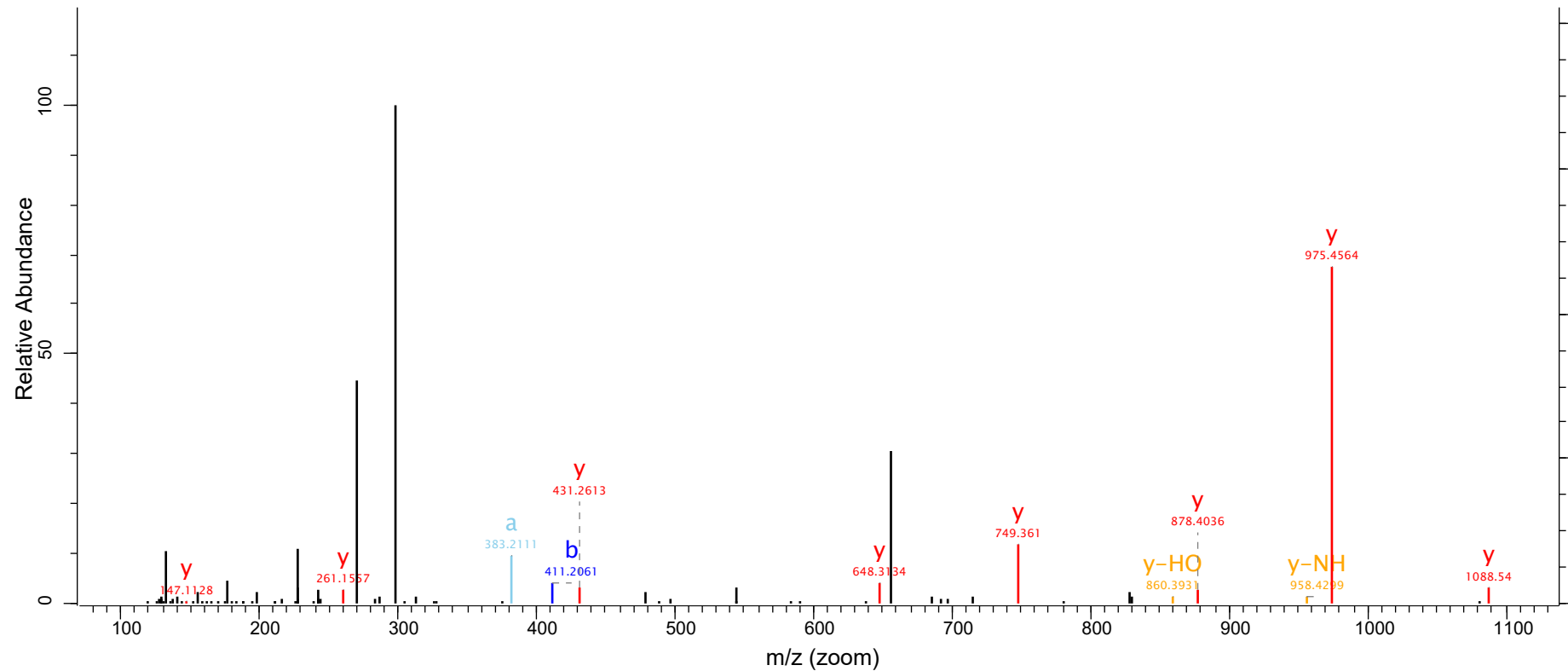
Scan
18976

Method
FTMS; HCD

Score
80.24

m/z
693.33

Gene names
RFTN1



Raw File
agg_pep_sort_pos_2

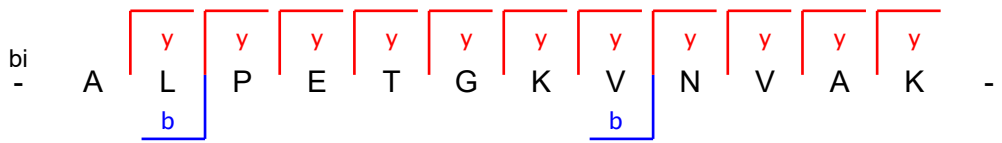
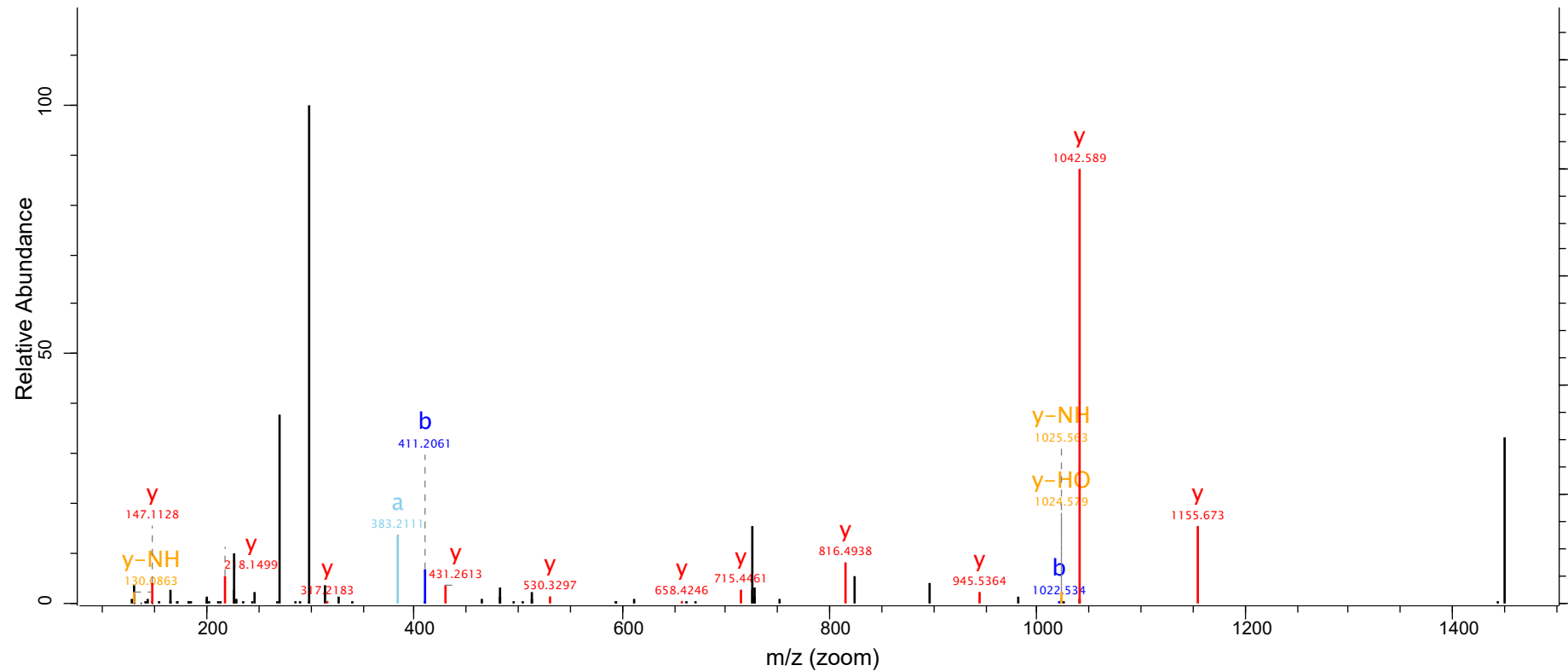
Scan
14730

Method
FTMS; HCD

Score
96.14

m/z
727.4

Gene names
RIOK2



Raw File
agg_pep_sort_pos_1

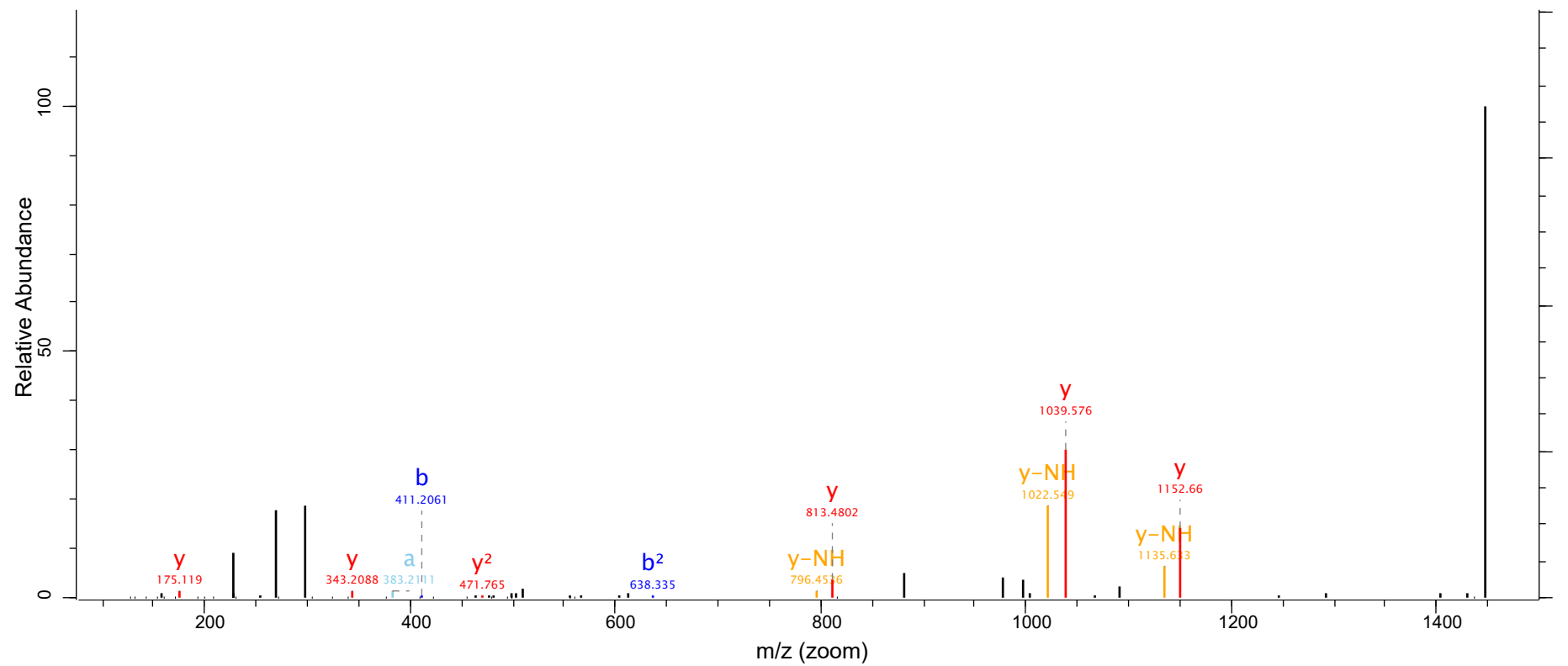
Scan
9890

Method
FTMS; HCD

Score
68.89

m/z
483.93

Gene names
RPL10L;RPL10



Raw File
agg_pep_sort_pos_3

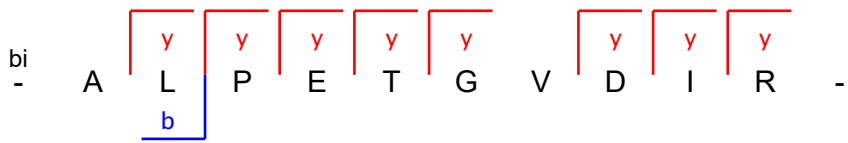
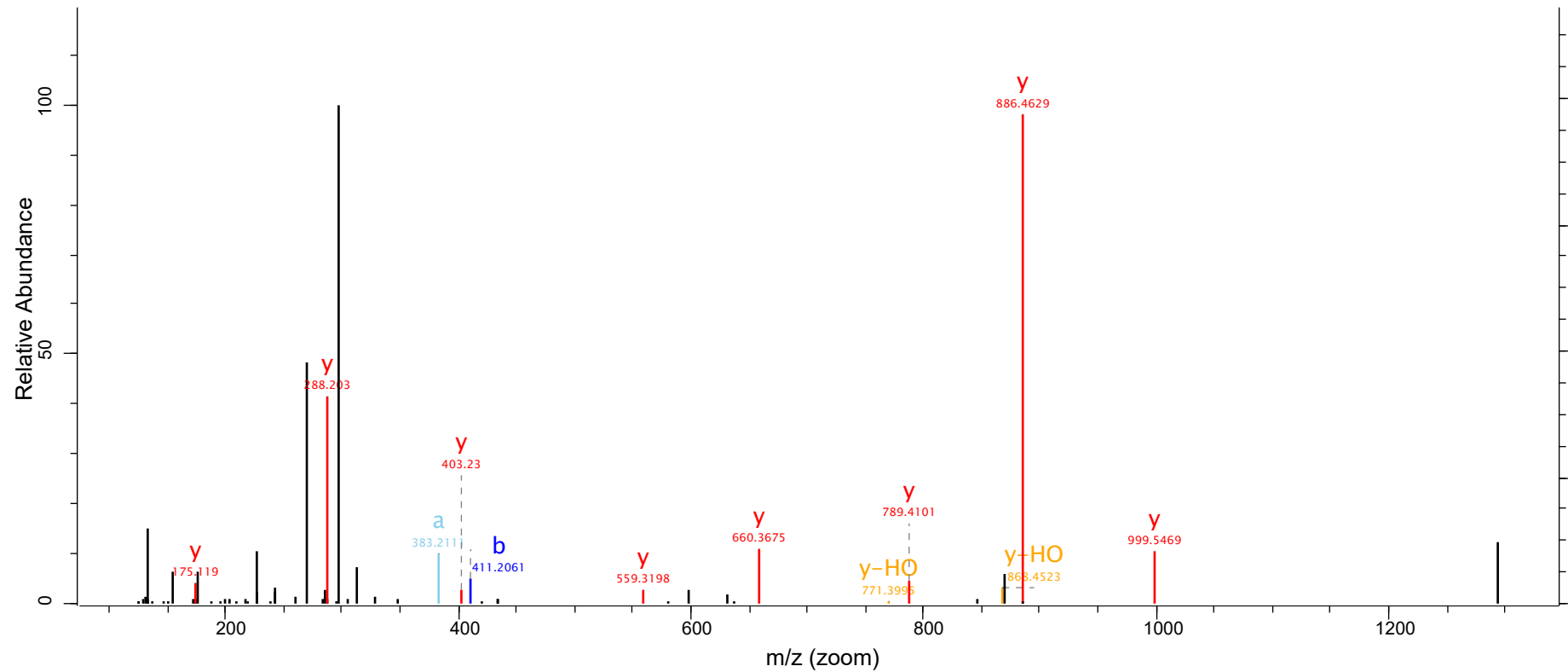
Scan
22319

Method
FTMS; HCD

Score
76.83

m/z
648.84

Gene names
RPL18



Raw File
agg_pep_sort_pos_2

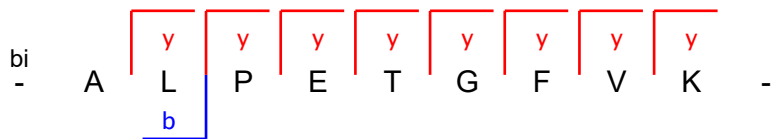
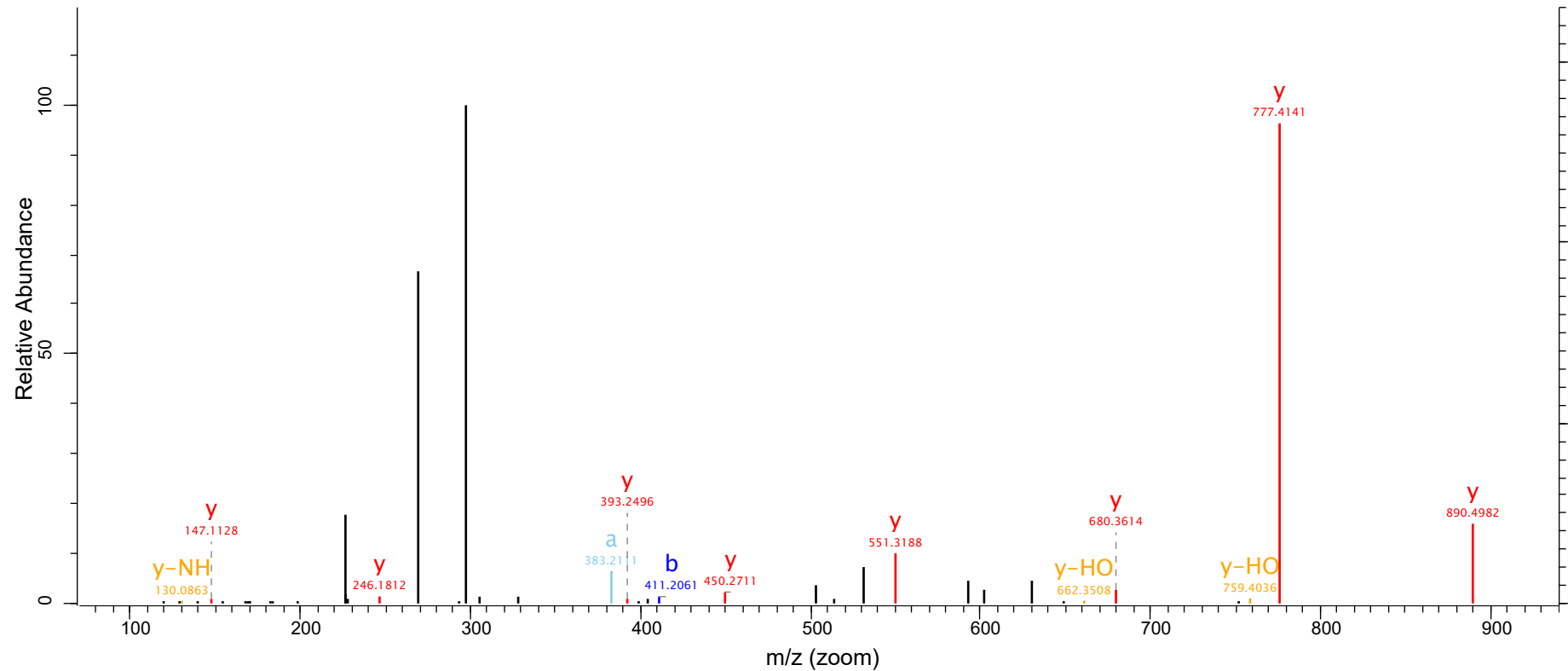
Scan
22697

Method
FTMS; HCD

Score
95.53

m/z
594.31

Gene names
RPL5



Raw File
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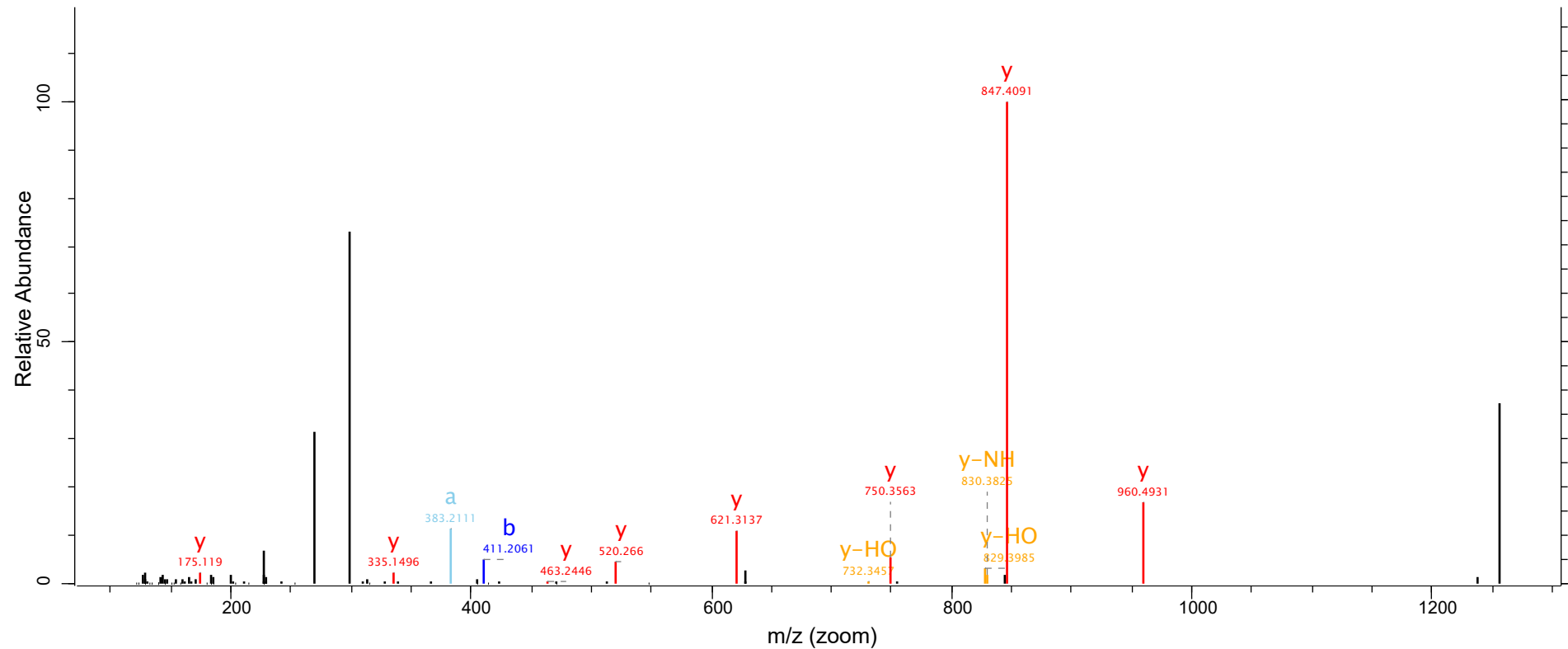
Scan
12082

Method
FTMS; HCD

Score
109.86

m/z
629.31

Gene names
RPS23



Raw File
agg_pep_sort_pos_2

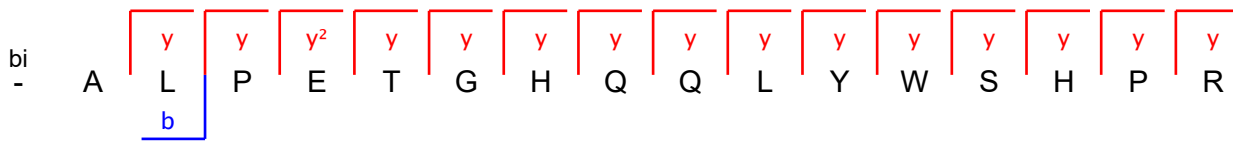
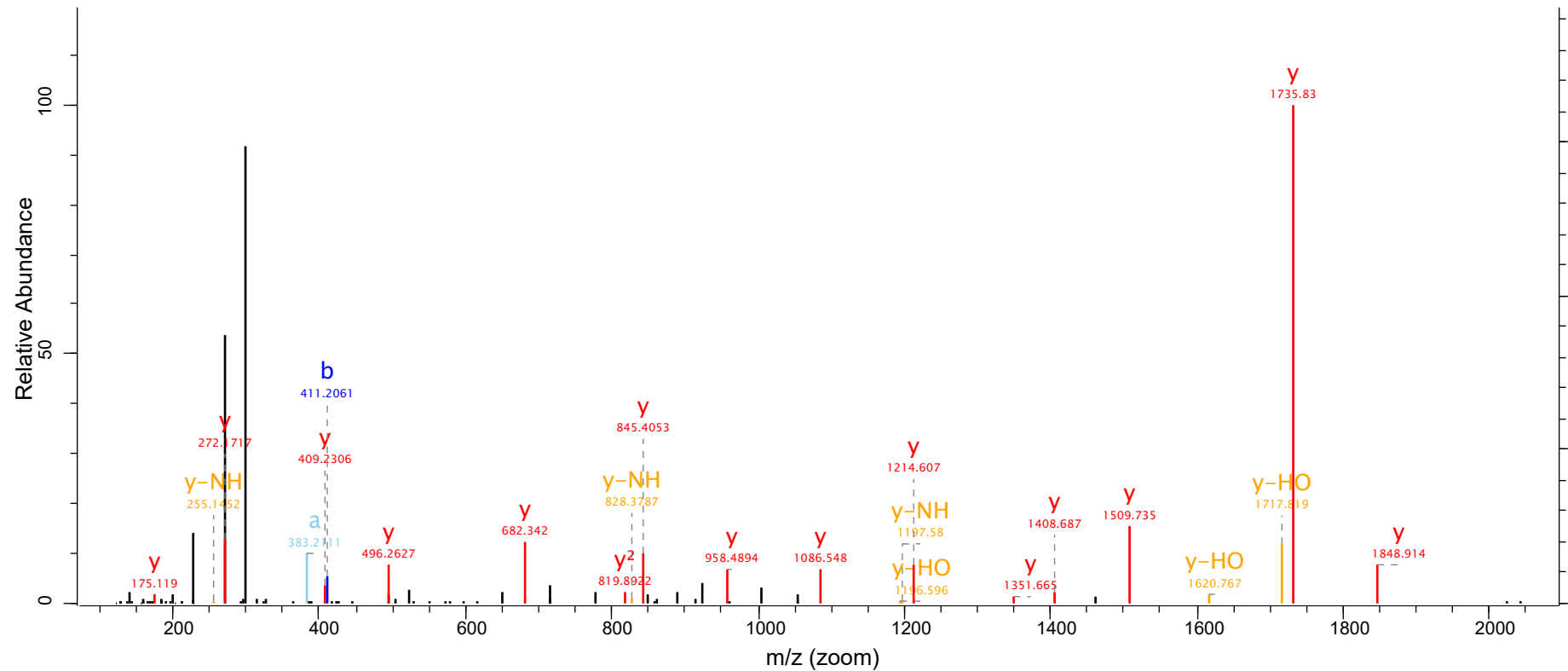
Scan
17042

Method
FTMS; HCD

Score
128.9

m/z
716.35

Gene names
RPS29



Raw File
agg_pep_sort_pos_2

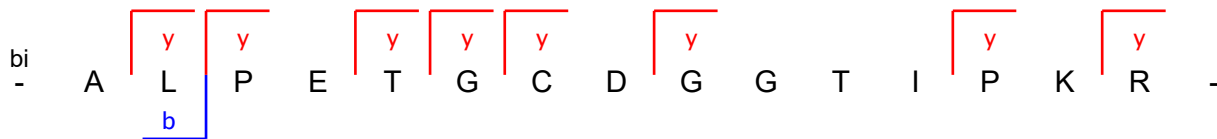
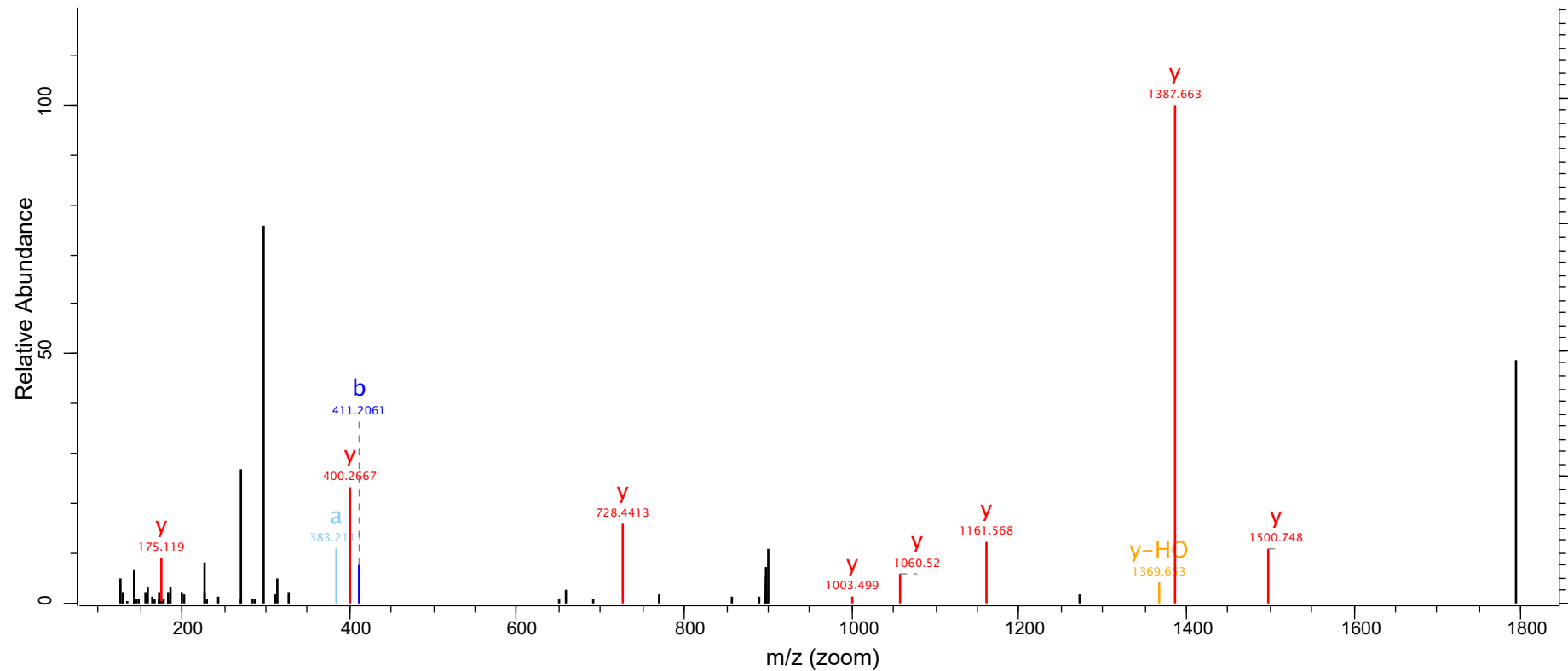
Scan
15731

Method
FTMS; HCD

Score
80.76

m/z
899.43

Gene names
RTFDC1



Raw File
agg_pep_sort_pos_2

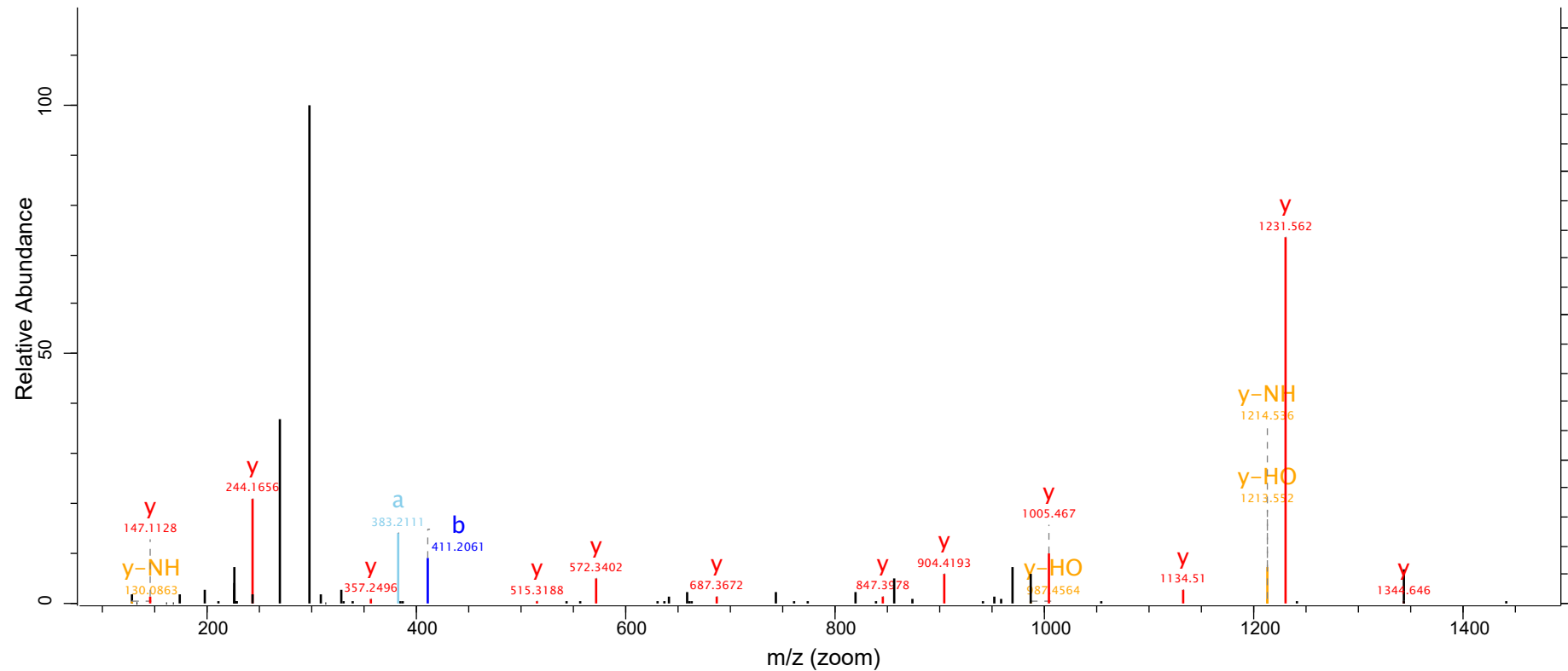
Scan
20000

Method
FTMS; HCD

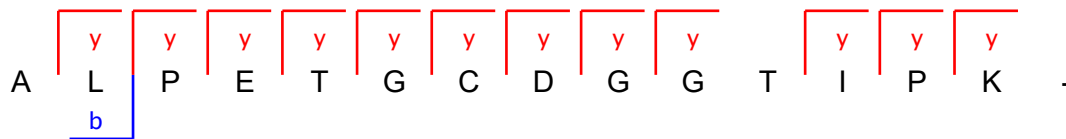
Score
113.63

m/z
821.38

Gene names
RTFDC1



bi



Raw File
agg_pep_sort_pos_1

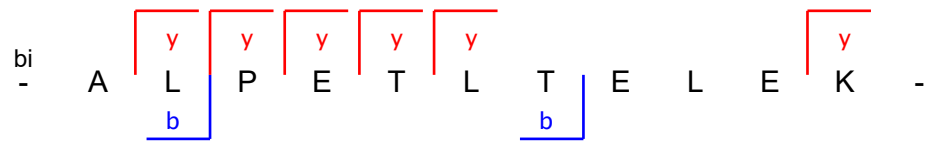
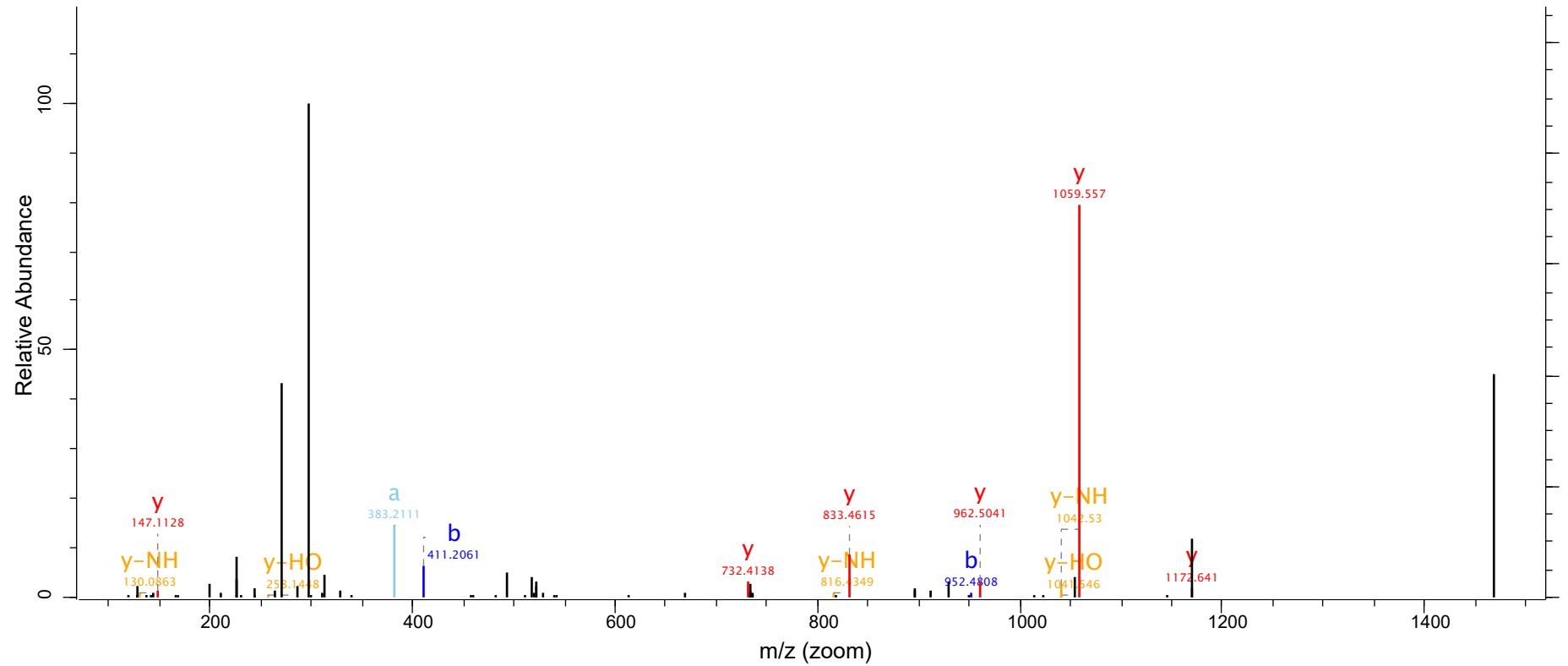
Scan
16795

Method
FTMS; HCD

Score
76.83

m/z
735.38

Gene names
S100A8



Raw File
agg_pep_sort_pos_2

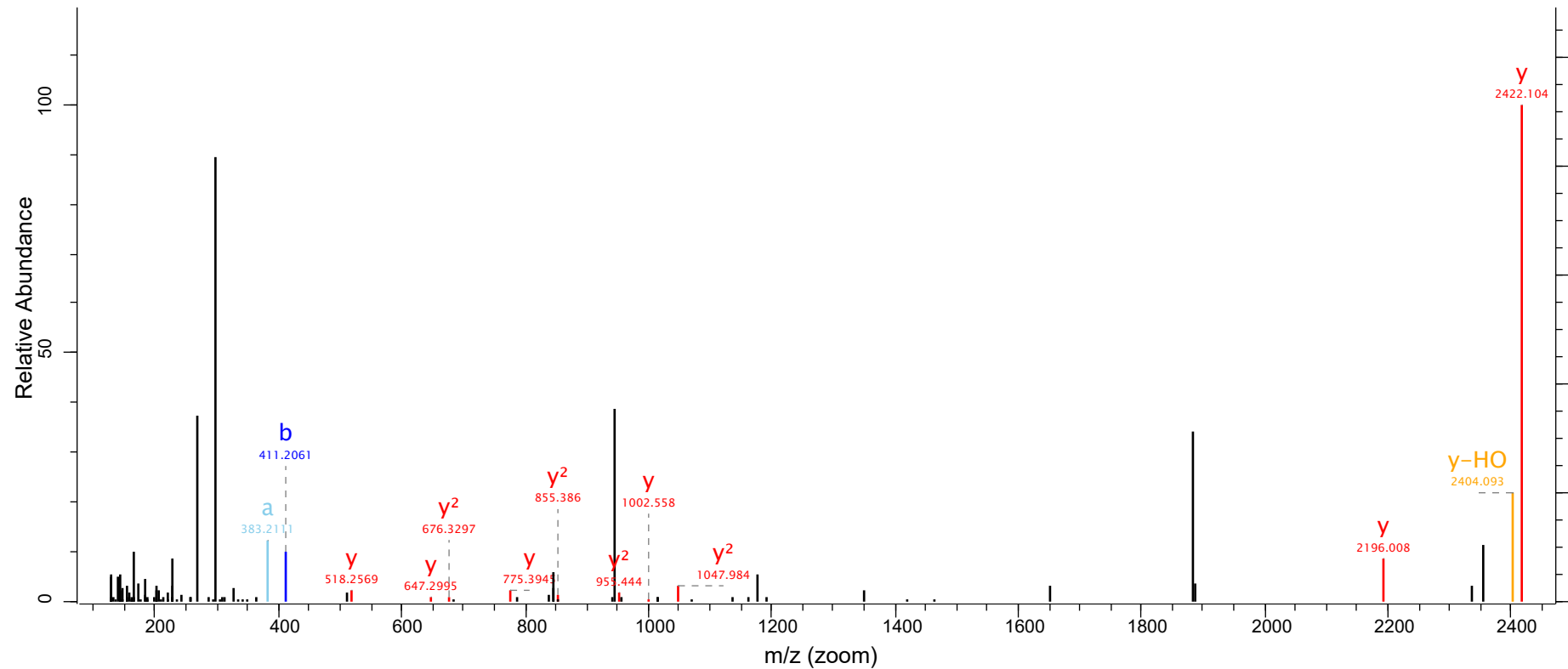
Scan
13126

Method
FTMS; HCD

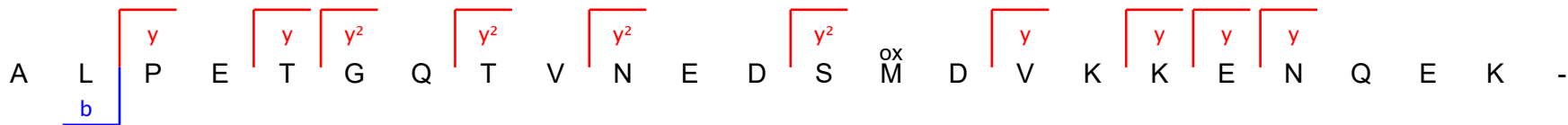
Score
48.66

m/z
945.11

Gene names
SCML2



bi



Raw File
agg_pep_sort_pos_2

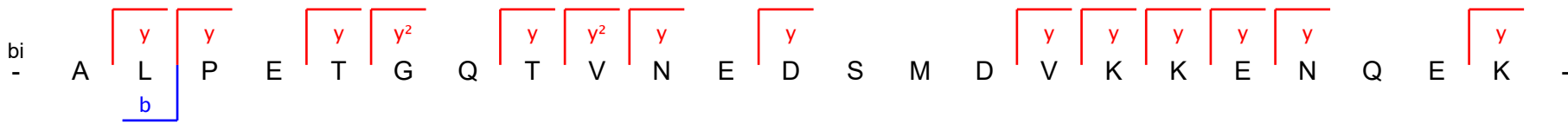
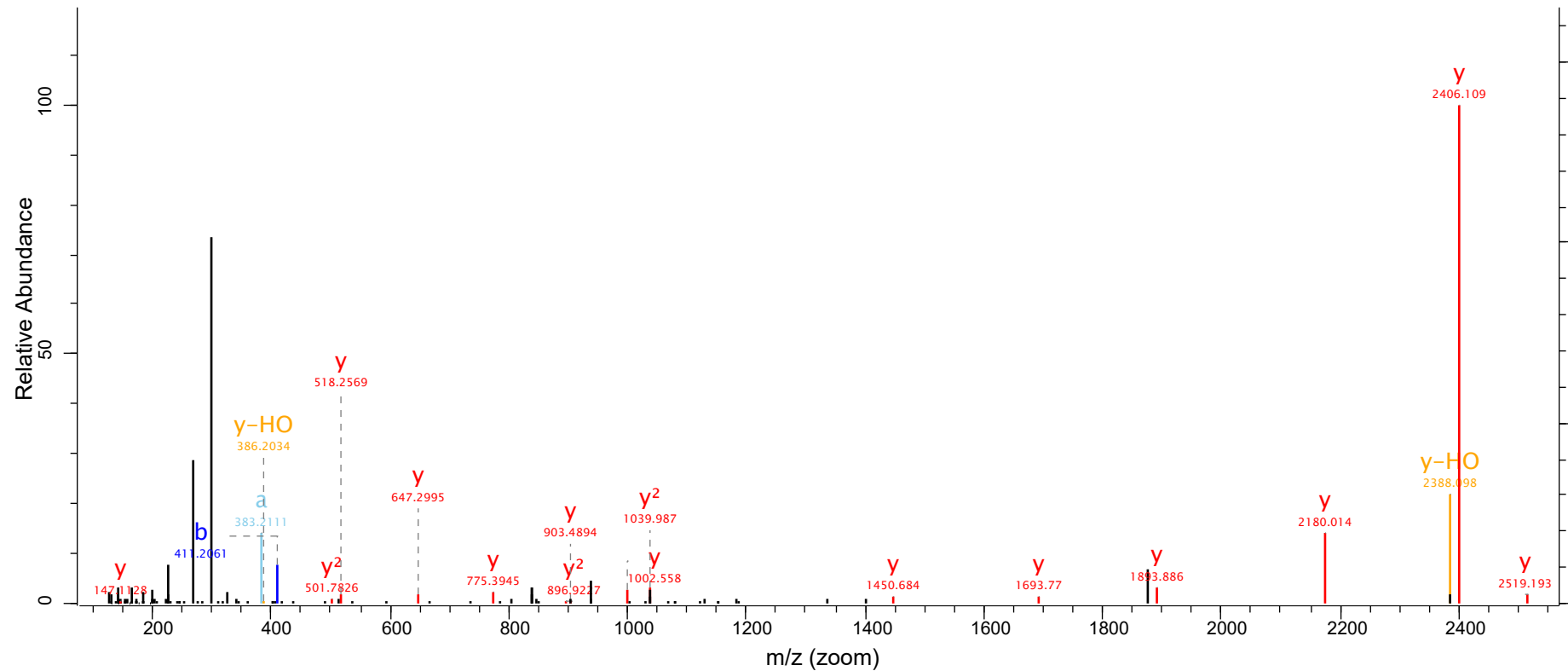
Scan
14590

Method
FTMS; HCD

Score
77.85

m/z
939.77

Gene names
SCML2



Raw File
agg_pep_sort_pos_2

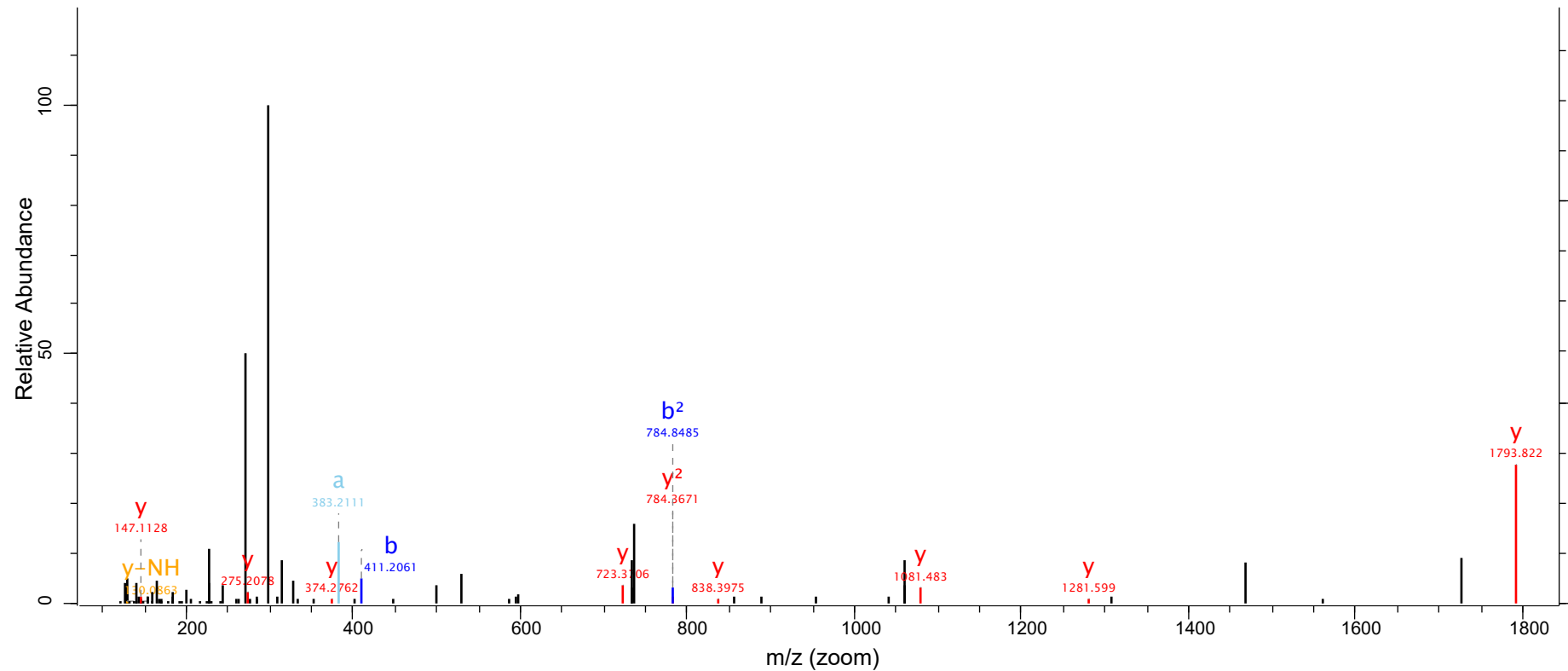
Scan
15363

Method
FTMS; HCD

Score
26.84

m/z
736.01

Gene names
SCML2



bi - A L P E T G Q T V N E D S ox M D V K K -

b

y

y²

y

y

y

y

y

y

y

b²

Raw File
agg_pep_sort_pos_3

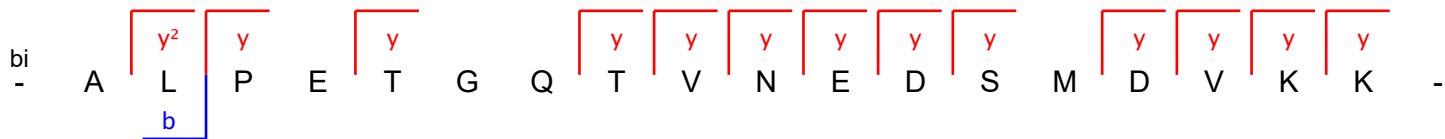
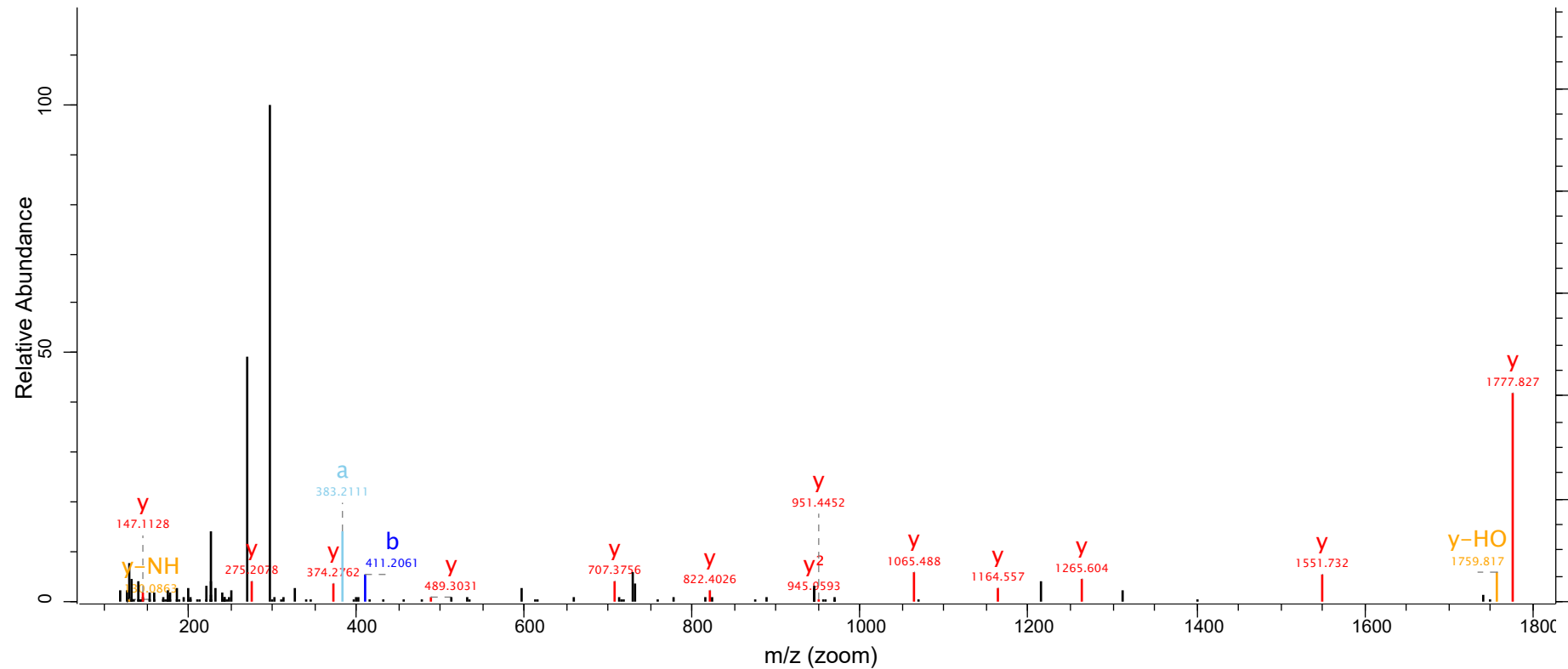
Scan
17179

Method
FTMS; HCD

Score
62.77

m/z
730.35

Gene names
SCML2



Raw File
agg_pep_sort_pos_1

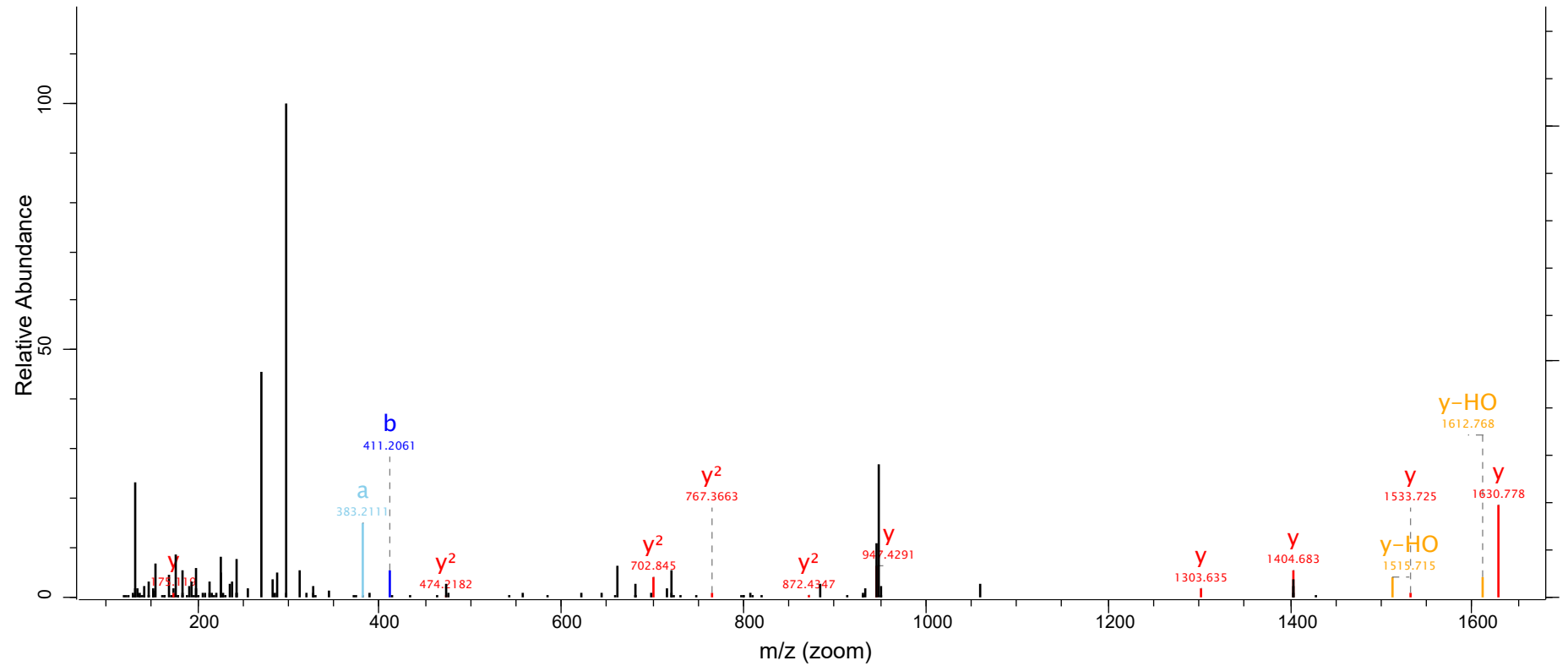
Scan
19823

Method
FTMS; HCD

Score
57.33

m/z
681.33

Gene names
SDR9C7



Raw File
agg_pep_sort_pos_1

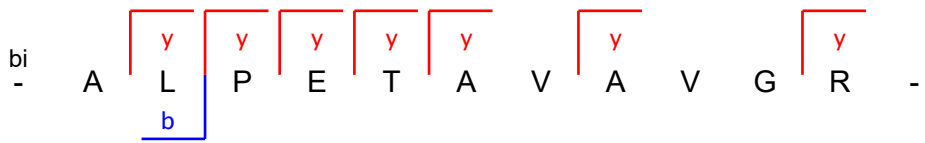
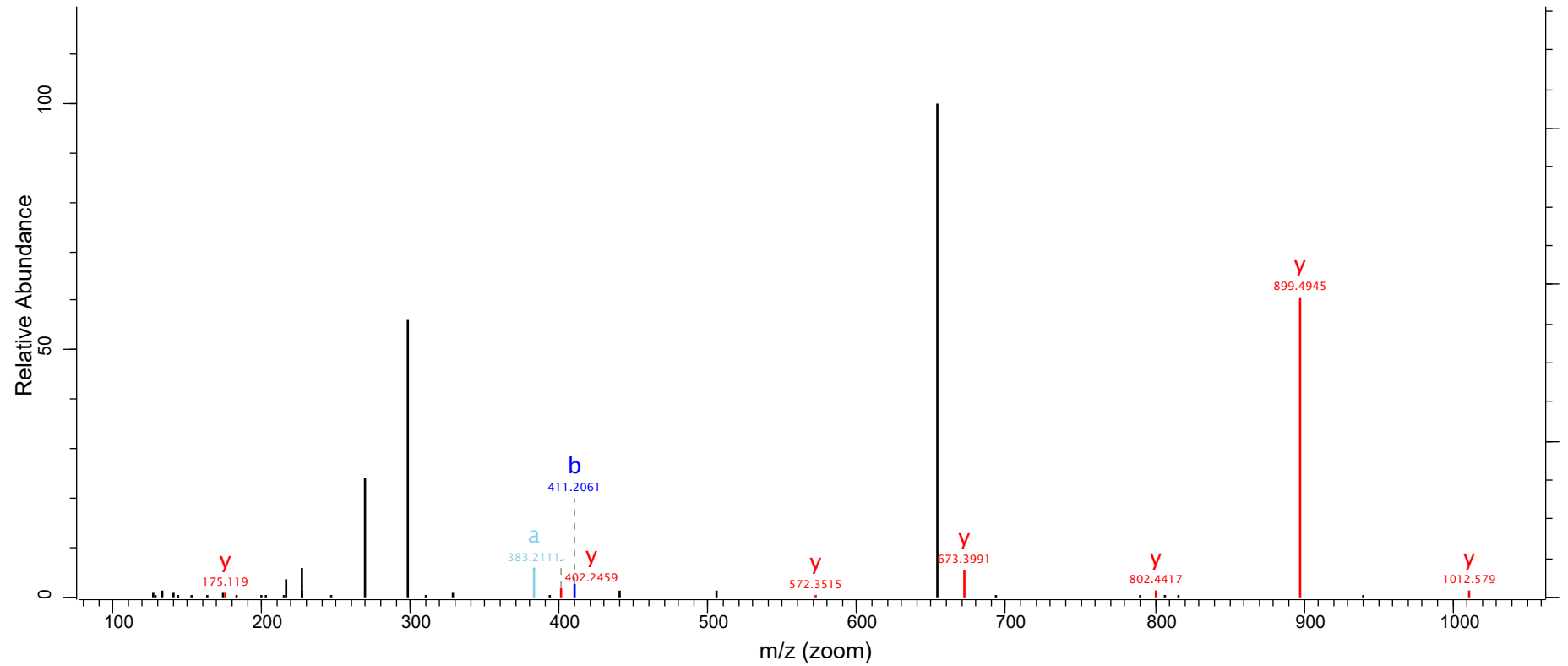
Scan
22938

Method
FTMS; HCD

Score
66.62

m/z
655.35

Gene names
SEPT11



Raw File
agg_pep_sort_pos_1

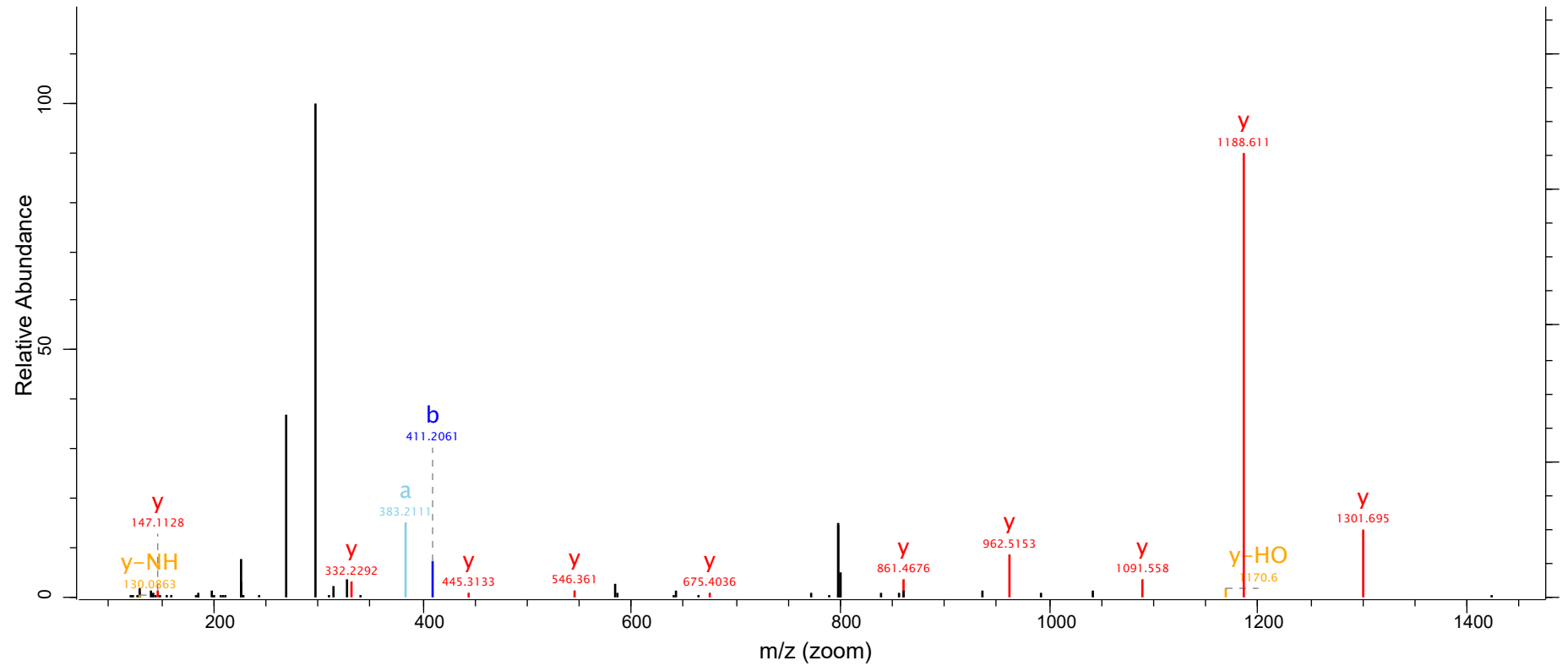
Scan
15979

Method
FTMS; HCD

Score
94.3

m/z
799.91

Gene names
SH3KBP1



Raw File
agg_pep_sort_pos_2

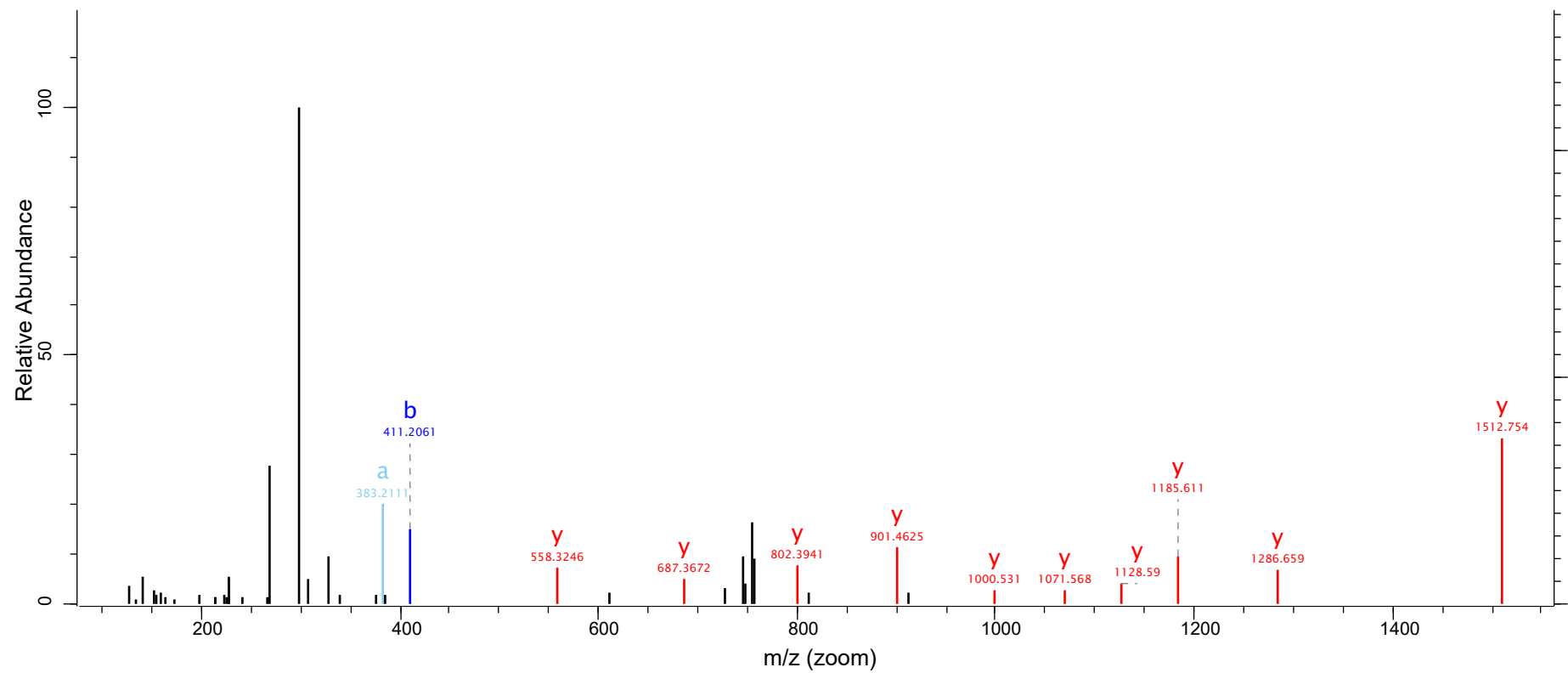
Scan
21675

Method
FTMS; HCD

Score
80.52

m/z
962.48

Gene names
SLC16A3



bi - A L P E T G G A V V D E G P T G V K -

Fragmentation diagram showing b and y ions. A blue bracket labeled 'b' spans from the start to the P residue. Red brackets labeled 'y' span from the P residue to the end of the peptide sequence.

Raw File
agg_pep_sort_pos_3

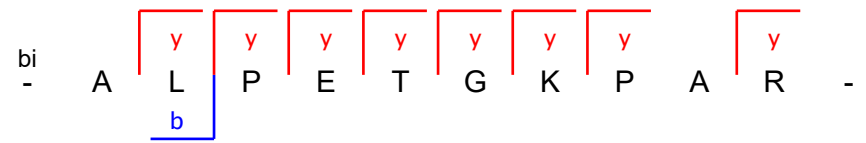
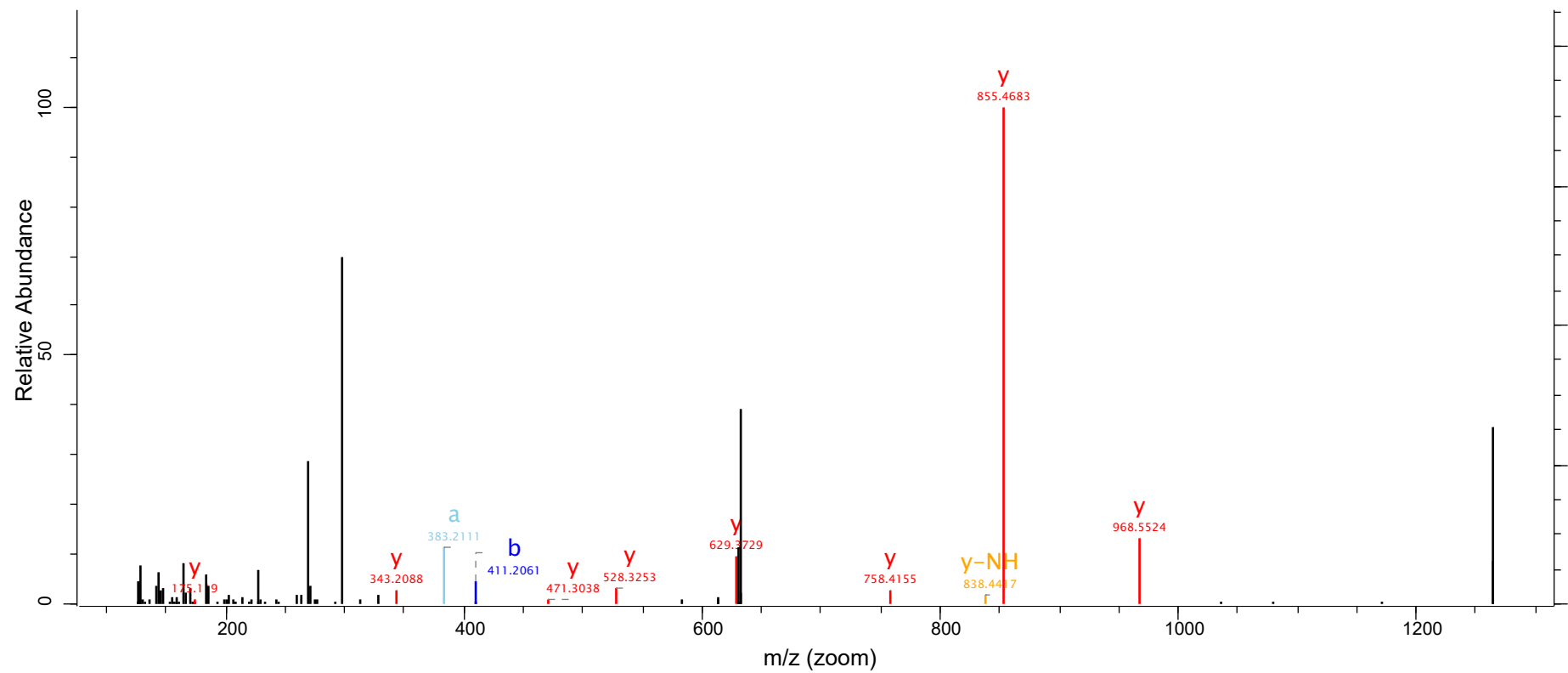
Scan
12803

Method
FTMS; HCD

Score
79.91

m/z
633.34

Gene names
SLC1A1



Raw File
agg_pep_sort_pos_2

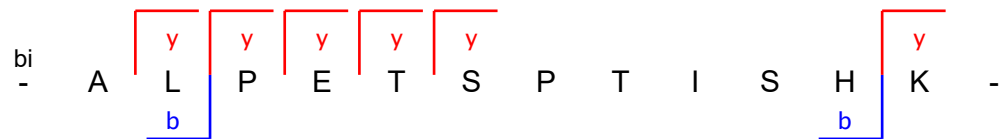
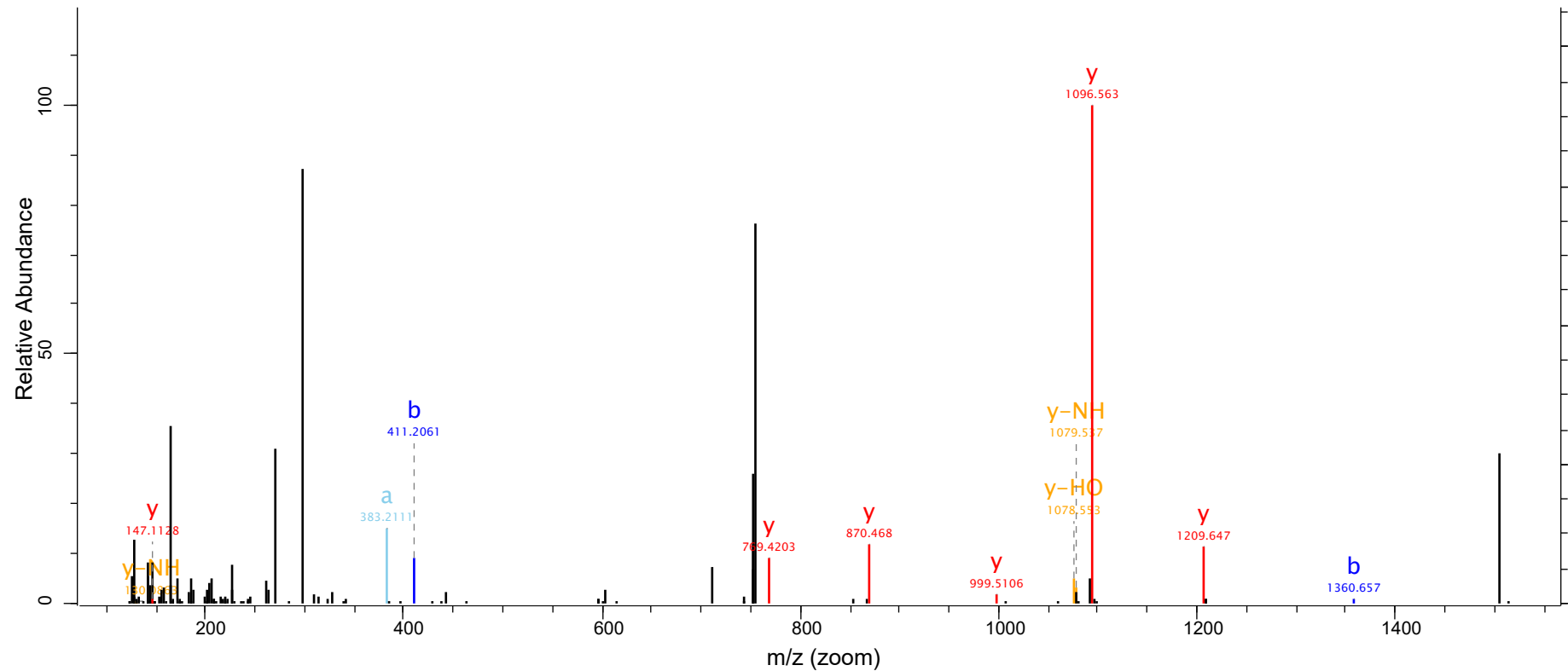
Scan
13478

Method
FTMS; HCD

Score
53.68

m/z
754.39

Gene names
SLC33A1



Raw File
agg_pep_sort_pos_1

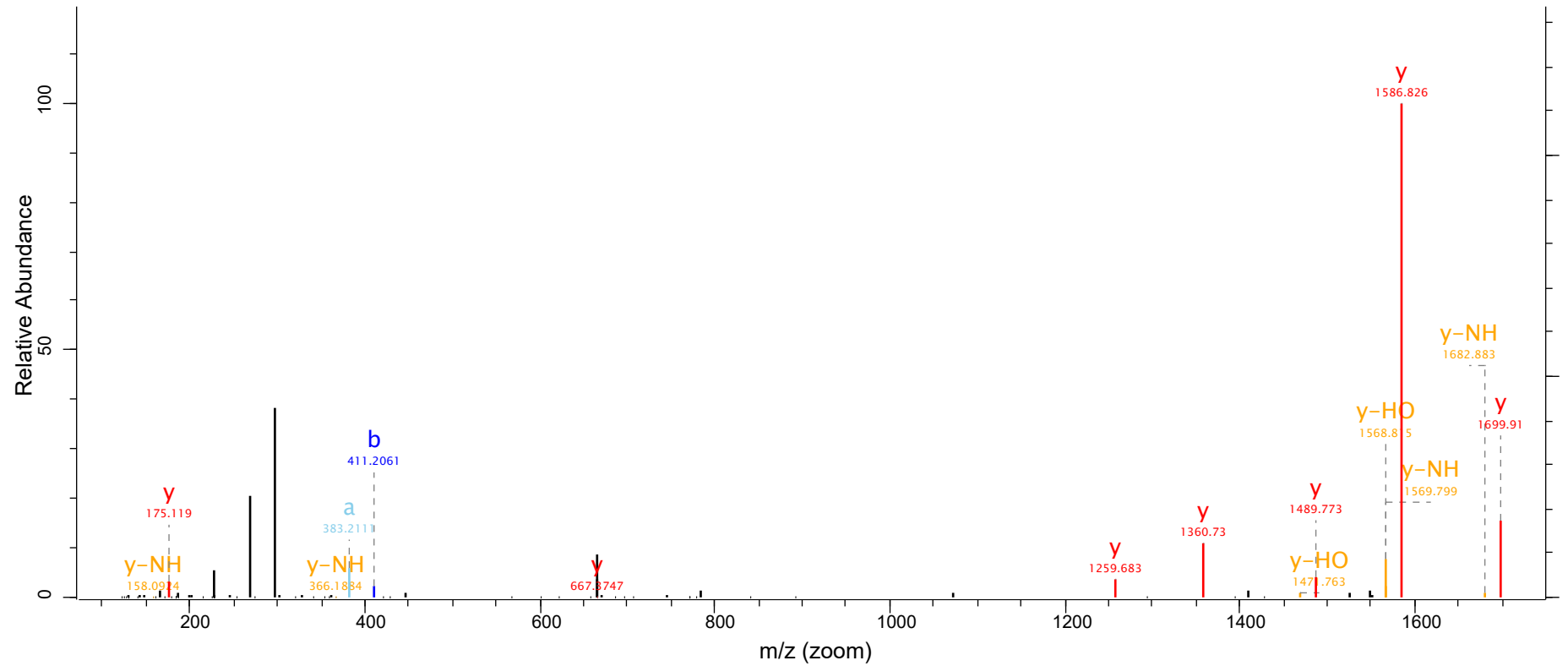
Scan
11877

Method
FTMS; HCD

Score
58.83

m/z
666.35

Gene names
SLC35D1



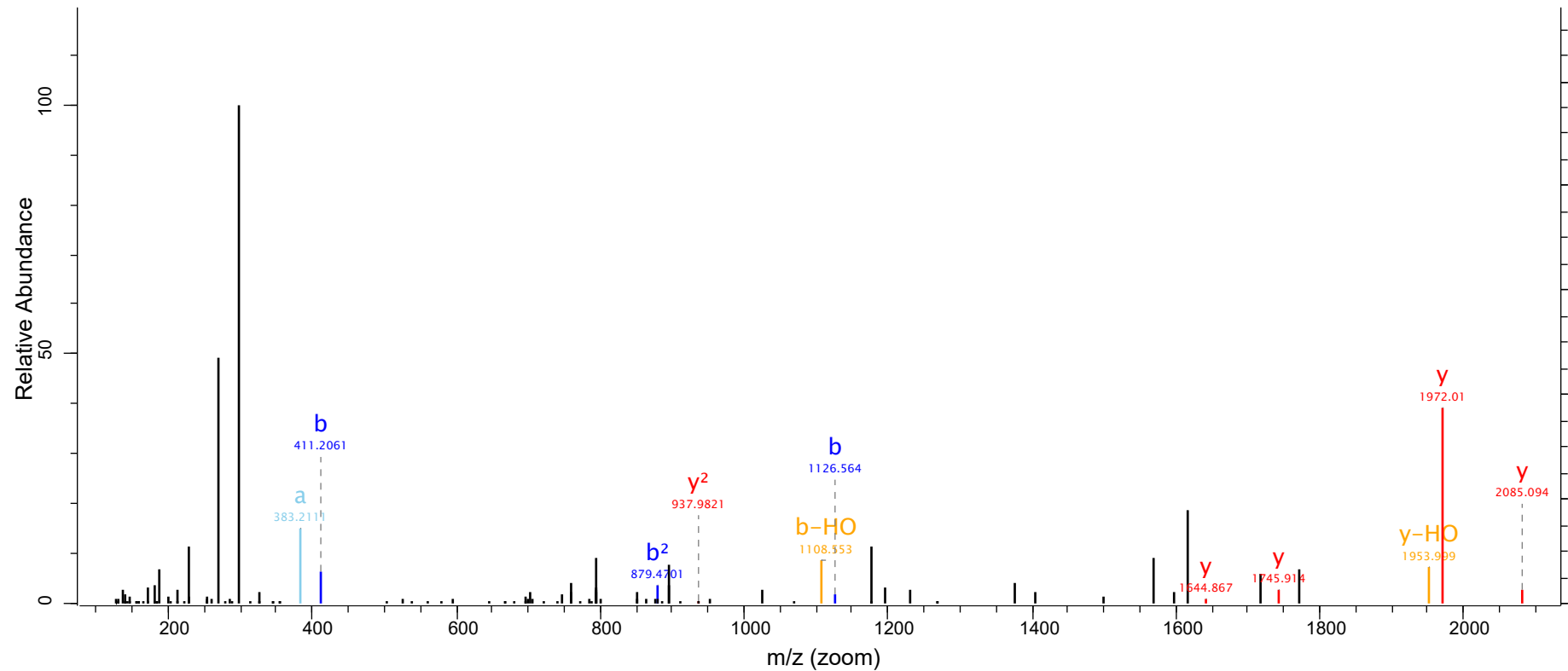
Raw File
agg_pep_sort_pos_2

Scan
16329

Method
FTMS; HCD

Score
44.57

m/z
795.08



Raw File
agg_pep_sort_pos_1

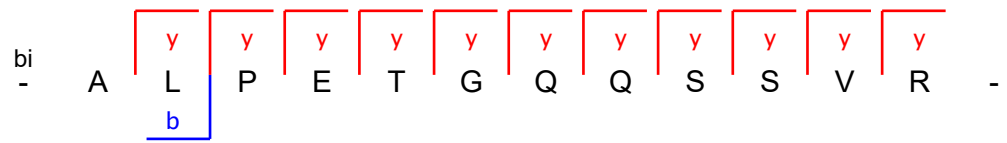
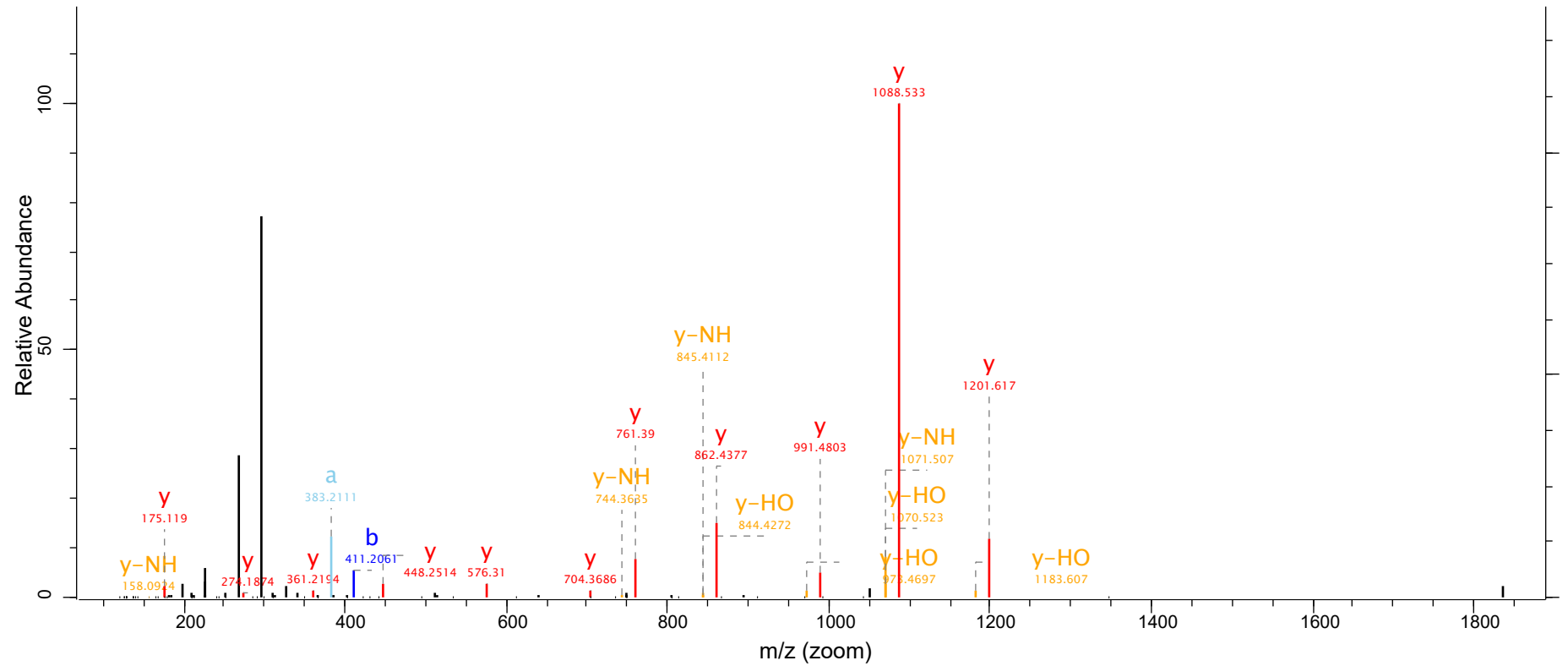
Scan
17536

Method
FTMS; HCD

Score
147.95

m/z
749.87

Gene names
SPANXB1



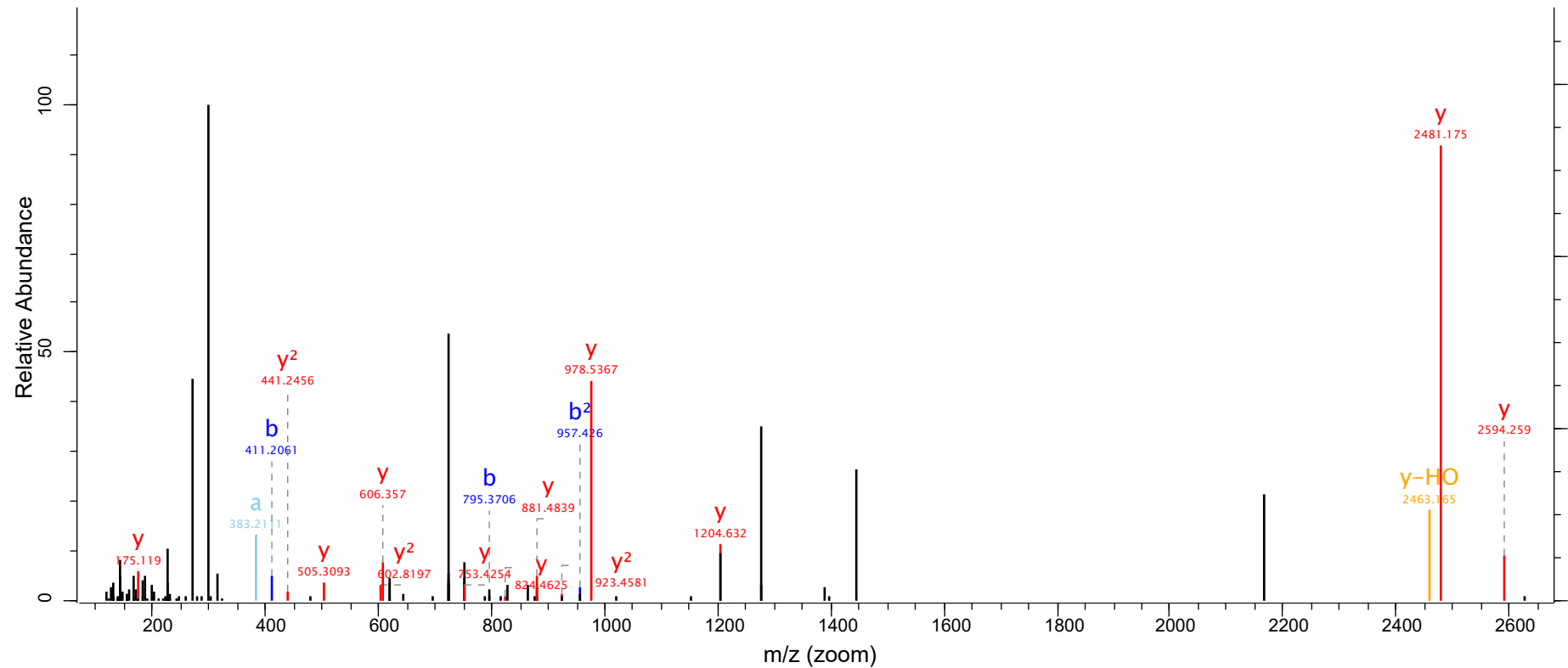
Raw File
agg_pep_sort_pos_3

Scan
13392

Method
FTMS; HCD

Score
66.38

m/z
724.1



Raw File
agg_pep_sort_pos_3

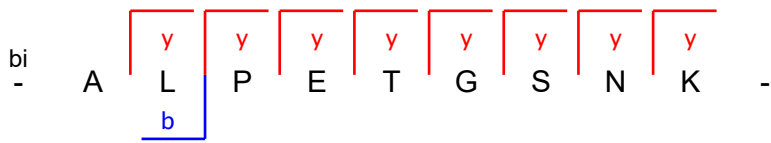
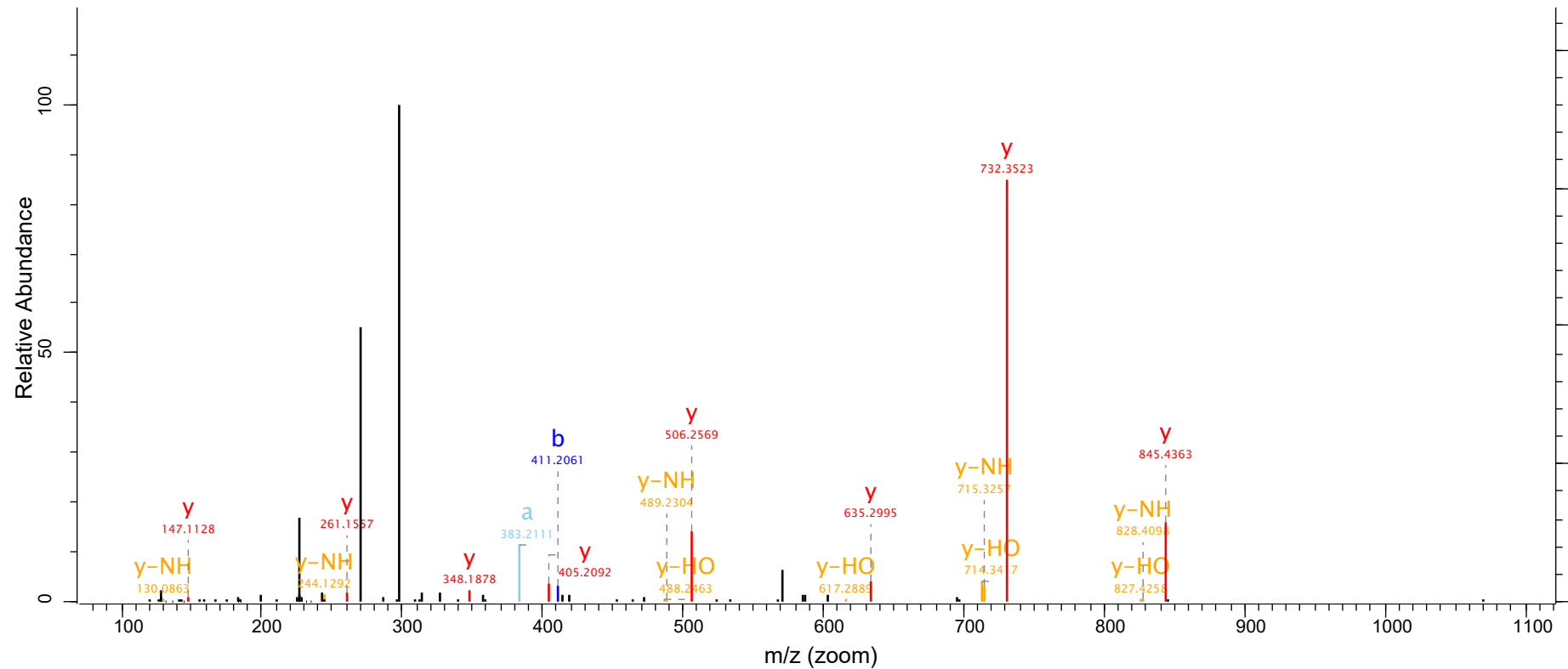
Scan
15899

Method
FTMS; HCD

Score
122.18

m/z
571.78

Gene names
SRC



Raw File
agg_pep_sort_pos_1

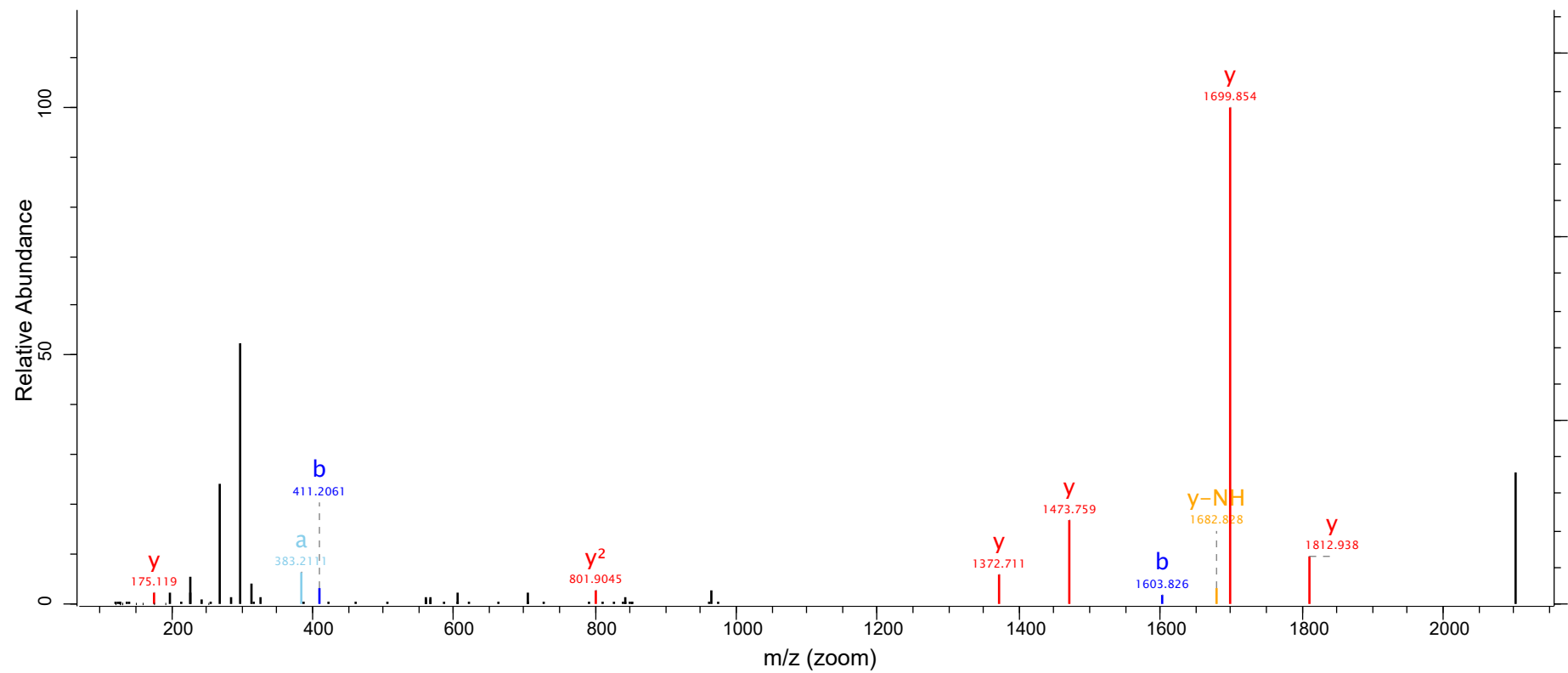
Scan
19096

Method
FTMS; HCD

Score
54.6

m/z
704.36

Gene names
STX2



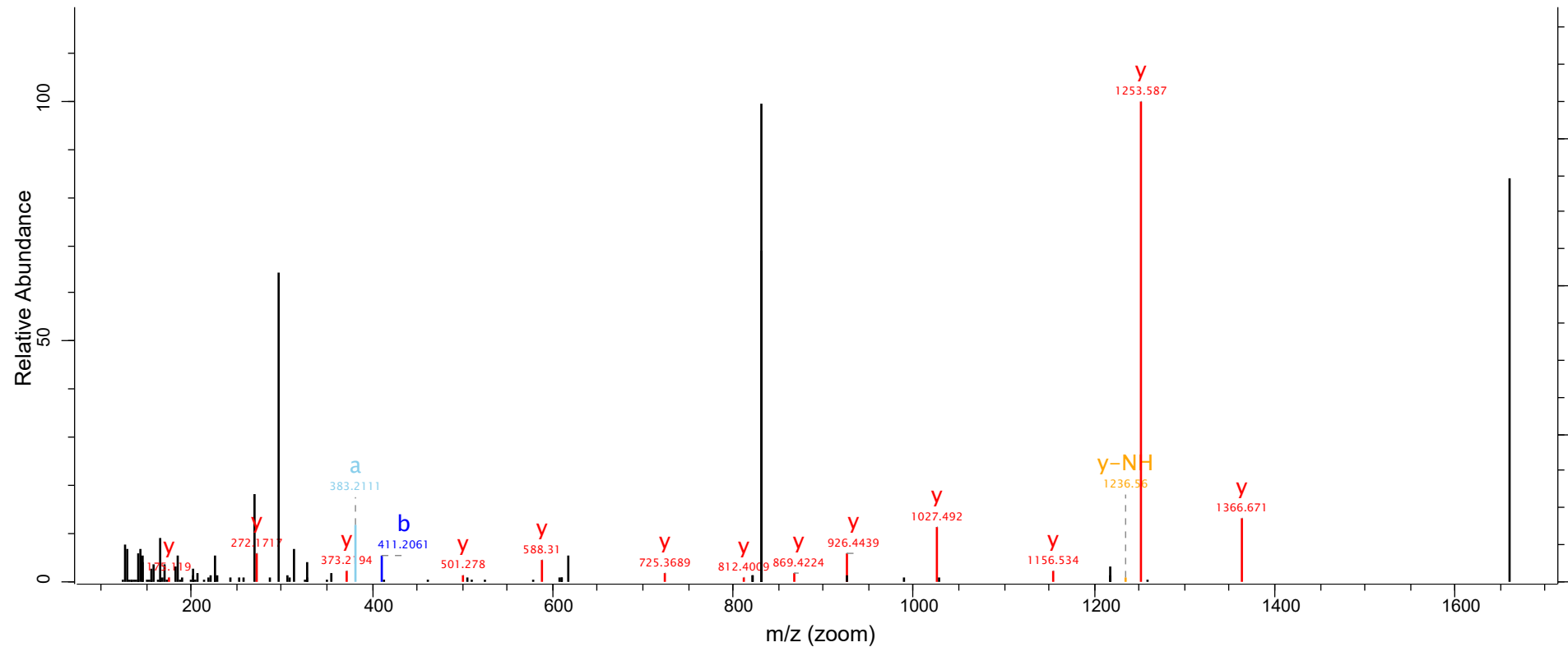
Raw File
agg_pep_sort_pos_1

Scan
12659

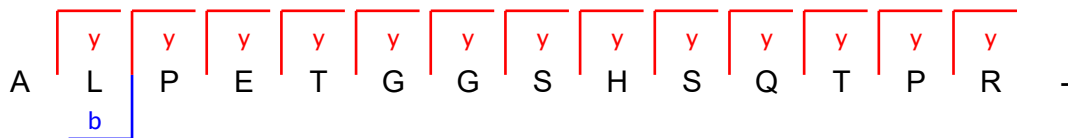
Method
FTMS; HCD

Score
72.29

m/z
832.4



bi



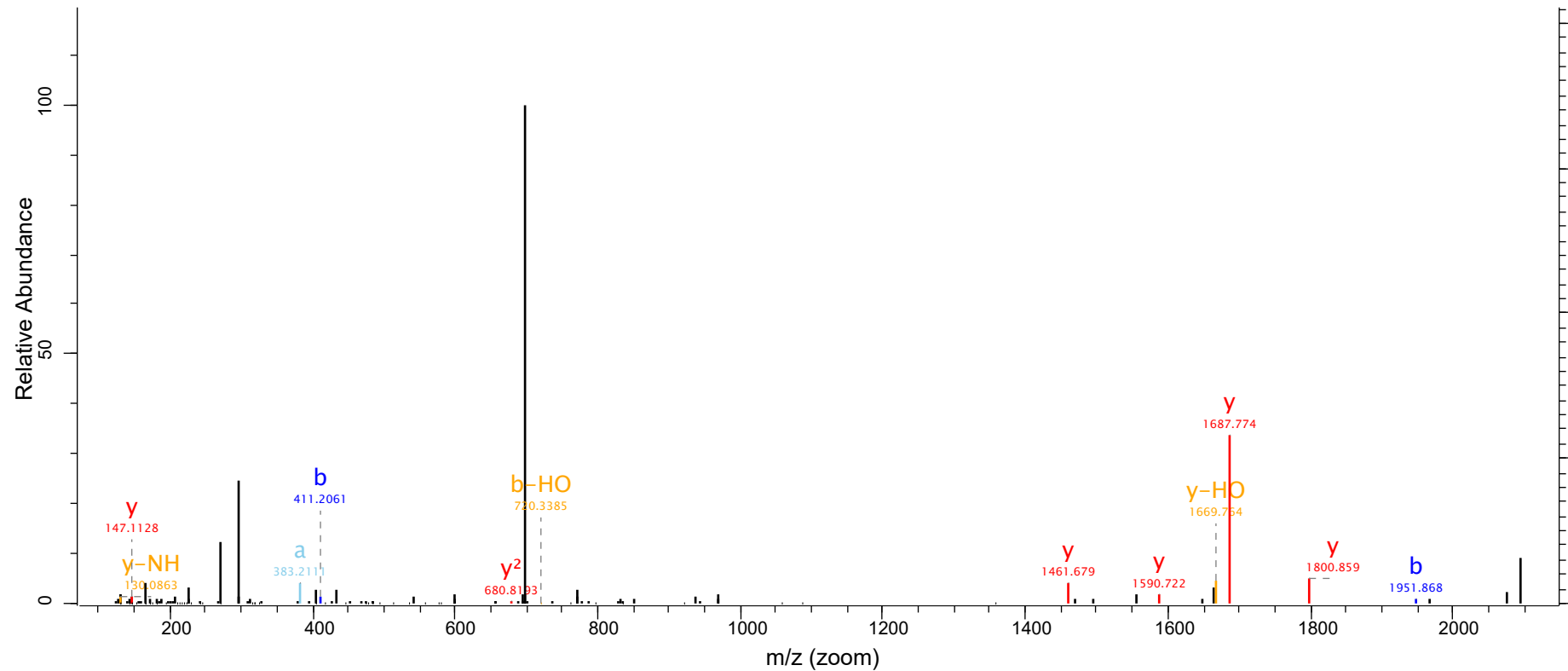
Raw File
agg_pep_sort_pos_1

Scan
9567

Method
FTMS; HCD

Score
38.38

m/z
700



Raw File
agg_pep_sort_pos_3

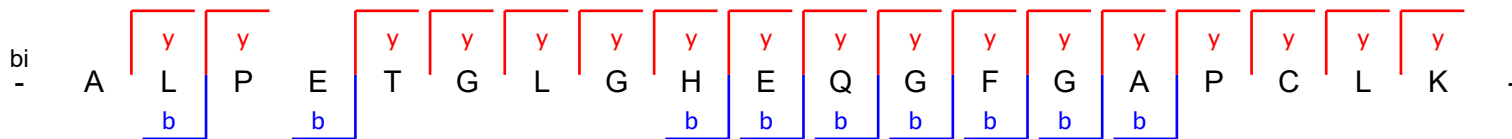
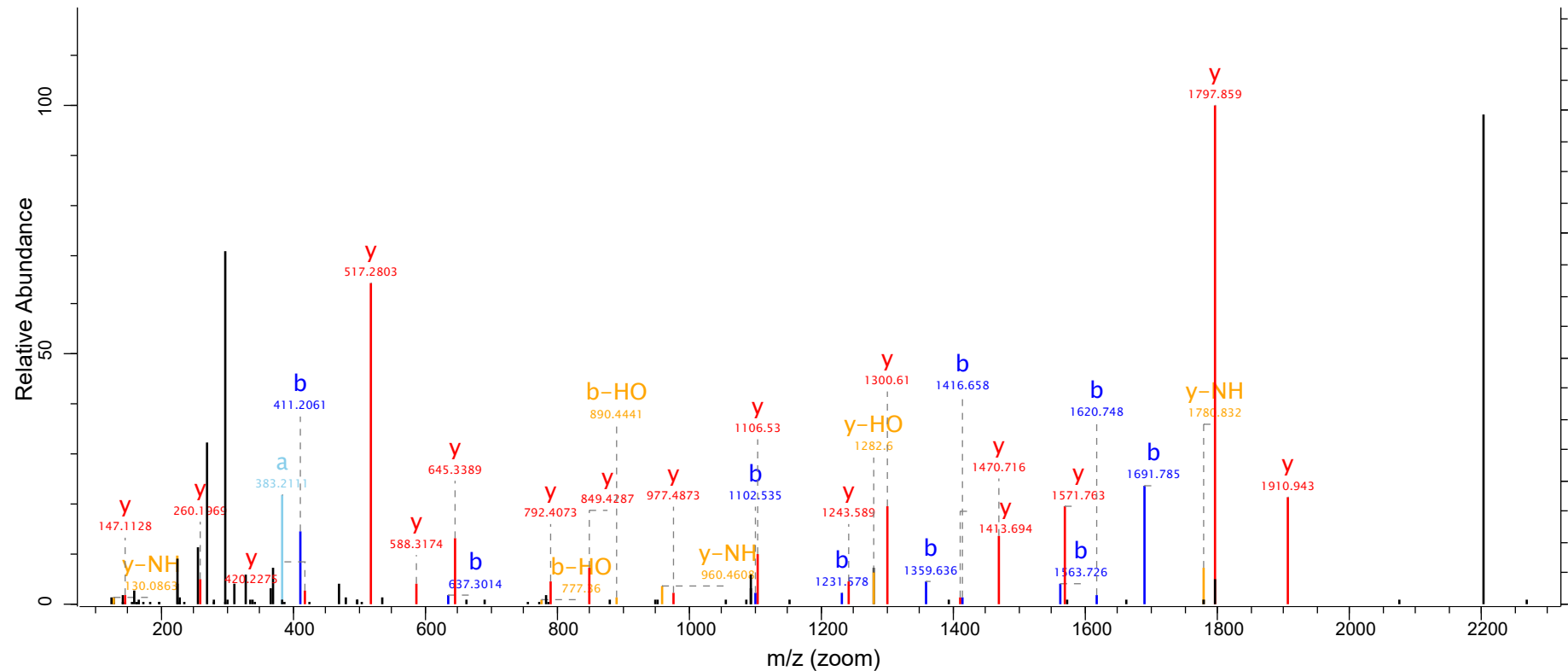
Scan
21147

Method
FTMS; HCD

Score
196.33

m/z
1105.04

Gene names
TES



Raw File
agg_pep_sort_pos_2

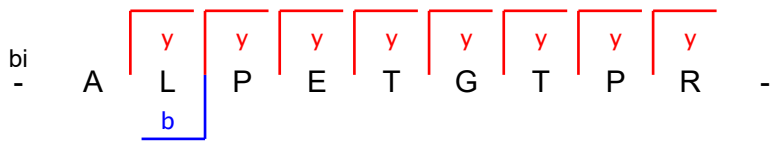
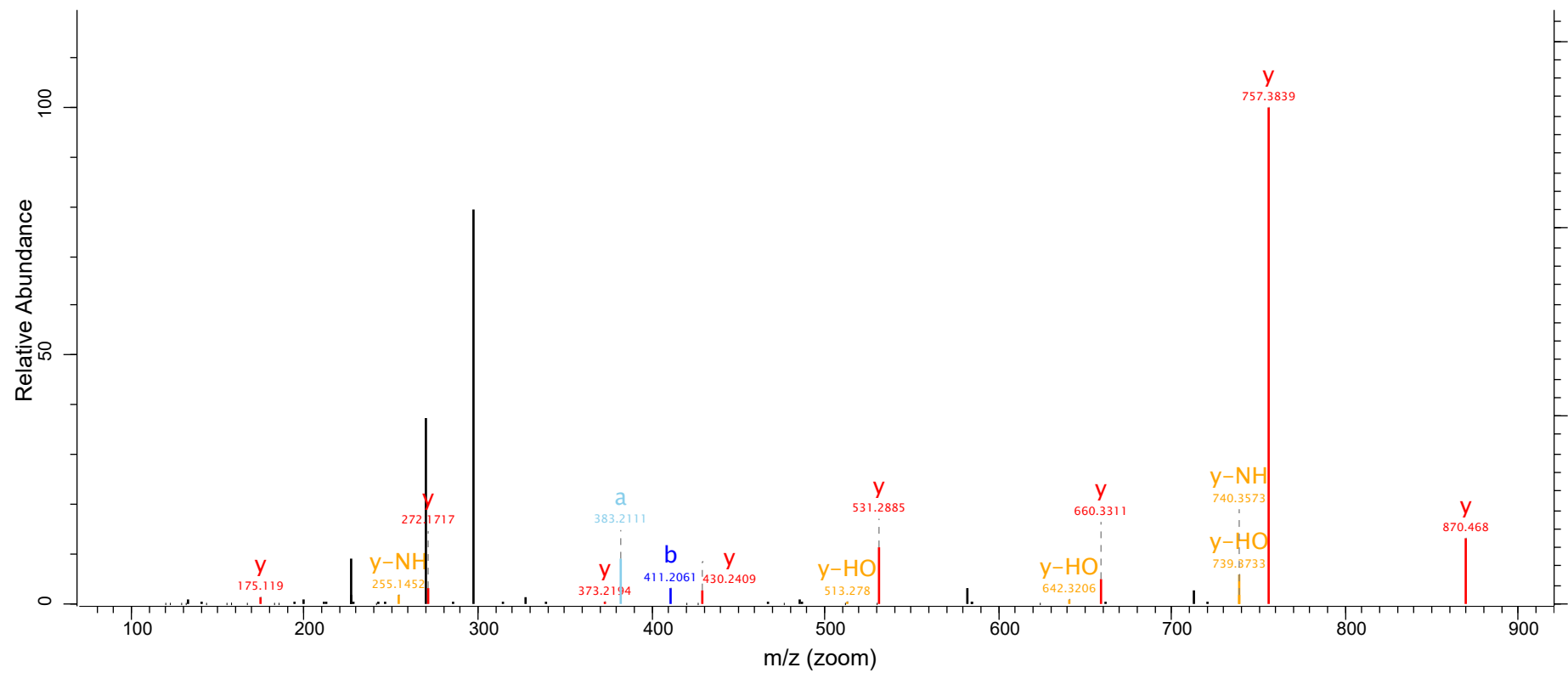
Scan
17865

Method
FTMS; HCD

Score
105.14

m/z
584.3

Gene names
TEX101;SEZ6L2



Raw File
agg_pep_sort_pos_1

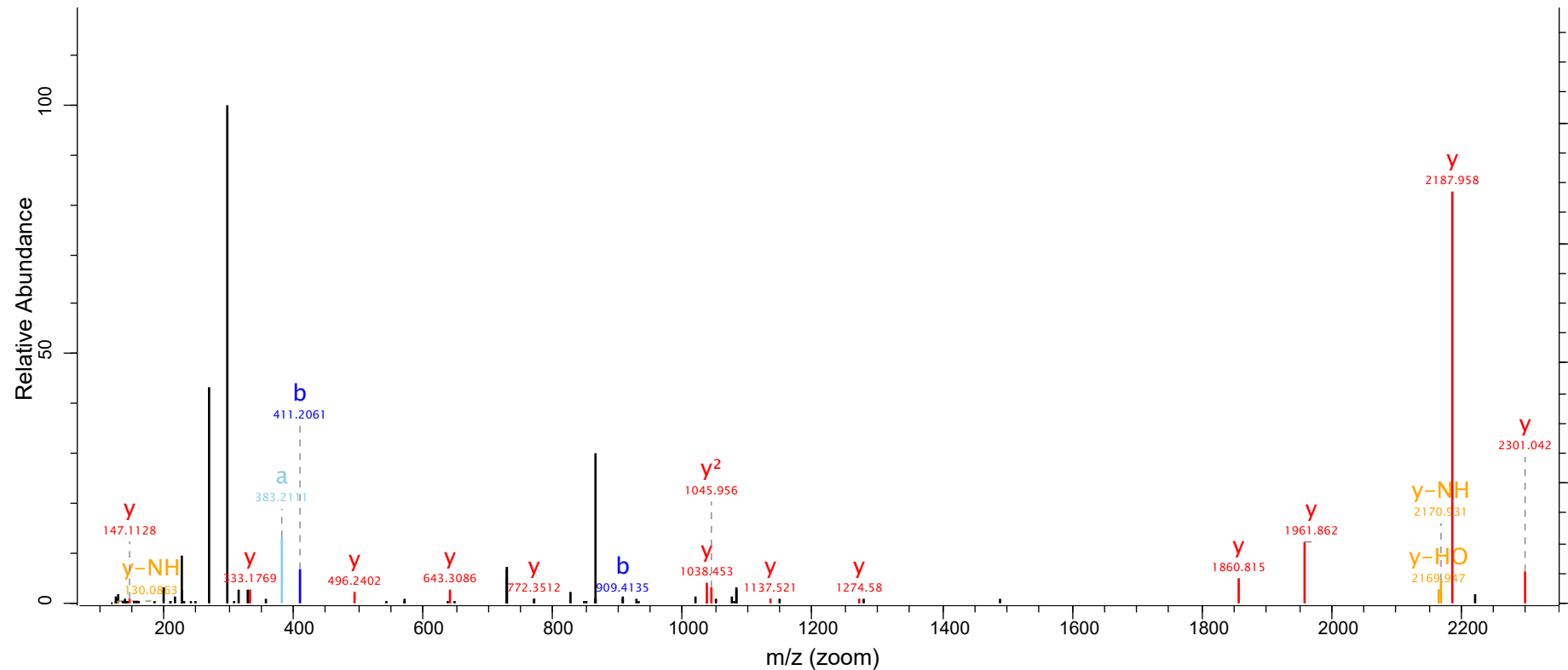
Scan
17360

Method
FTMS; HCD

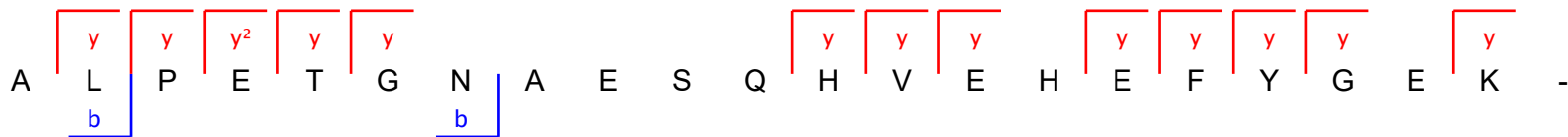
Score
62.19

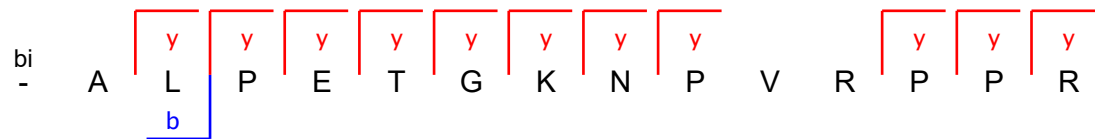
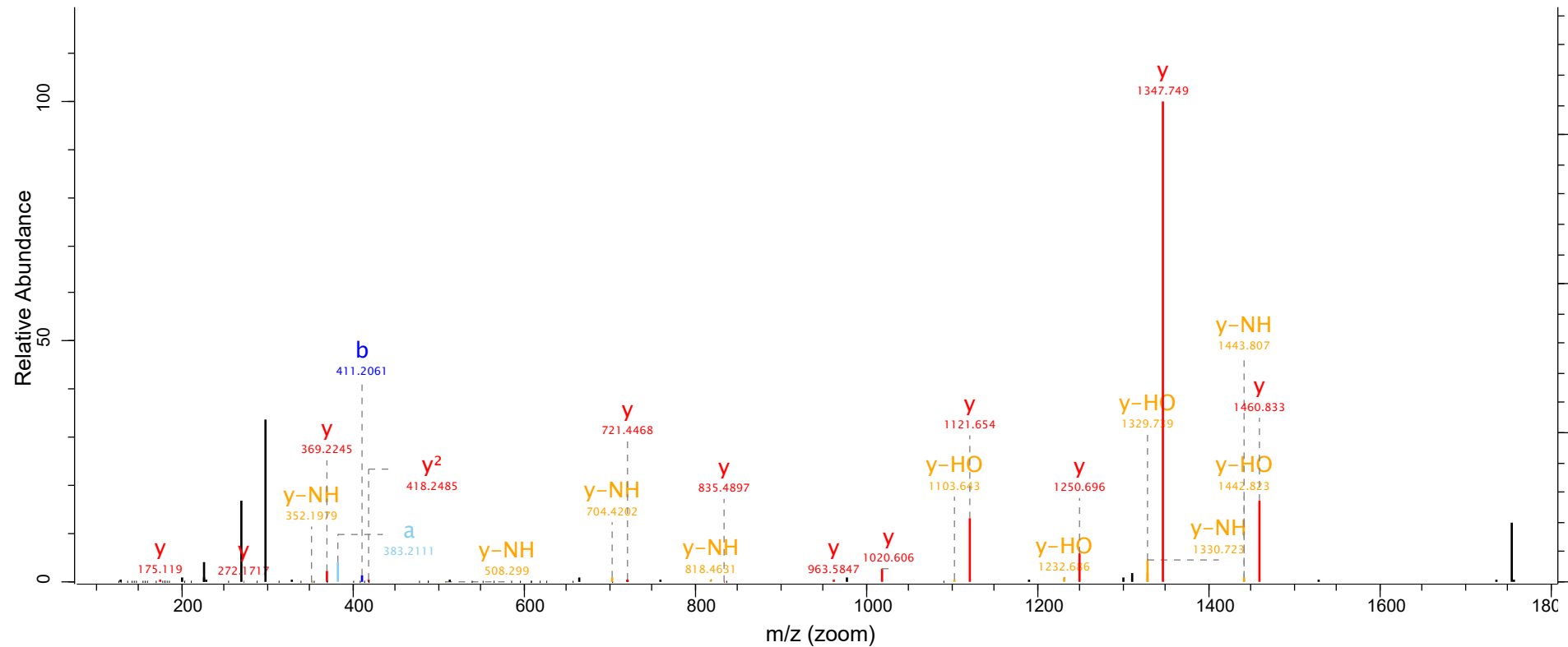
m/z
867.06

Gene names
TIAM1



bi





Raw File
agg_pep_sort_pos_3

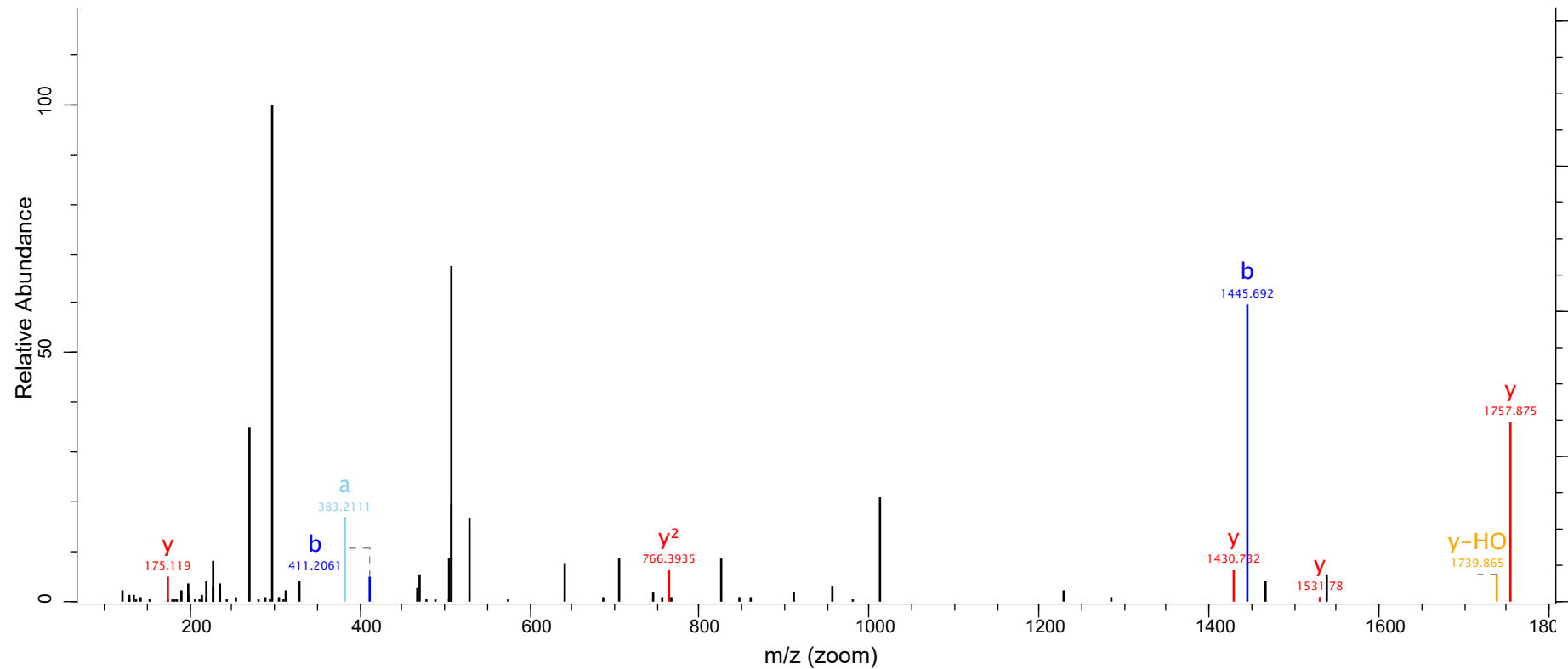
Scan
18153

Method
FTMS; HCD

Score
53.4

m/z
723.36

Gene names
TMPRSS11A



bi - A L P E T M Y R T V G F G T R S R -

Fragmentation diagram showing the sequence of amino acids: A L P E T M Y R T V G F G T R S R -. Red brackets indicate y-series fragments (L-P, E-T, T-M, S-R). Blue brackets indicate b-series fragments (L, V-G). The y-series fragments are labeled 'y' and the b-series fragments are labeled 'b'.

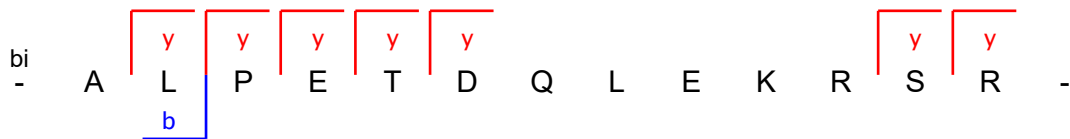
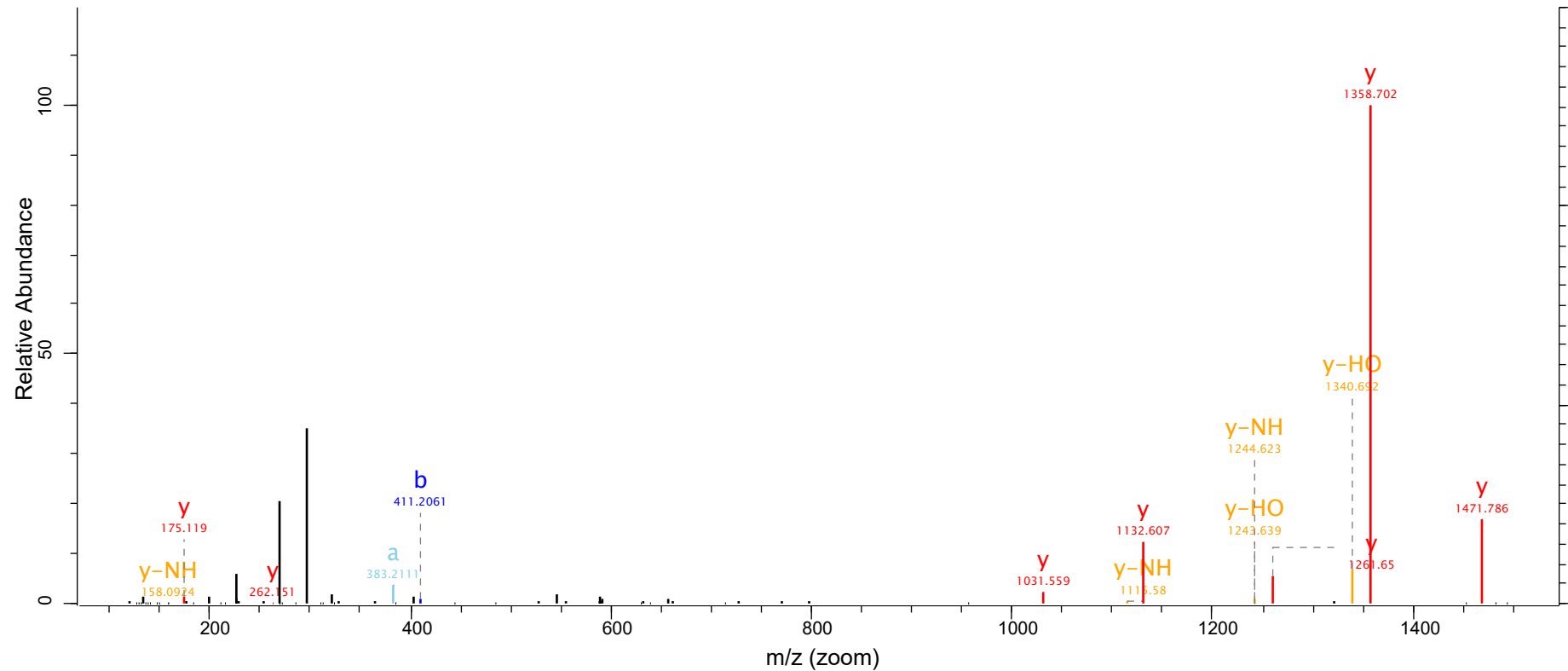
Raw File
agg_pep_sort_pos_3

Scan
16575

Method
FTMS; HCD

Score
65.56

m/z
590.31



Raw File
agg_pep_sort_pos_3

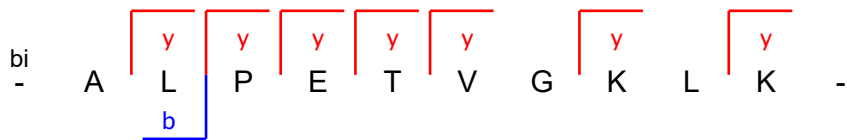
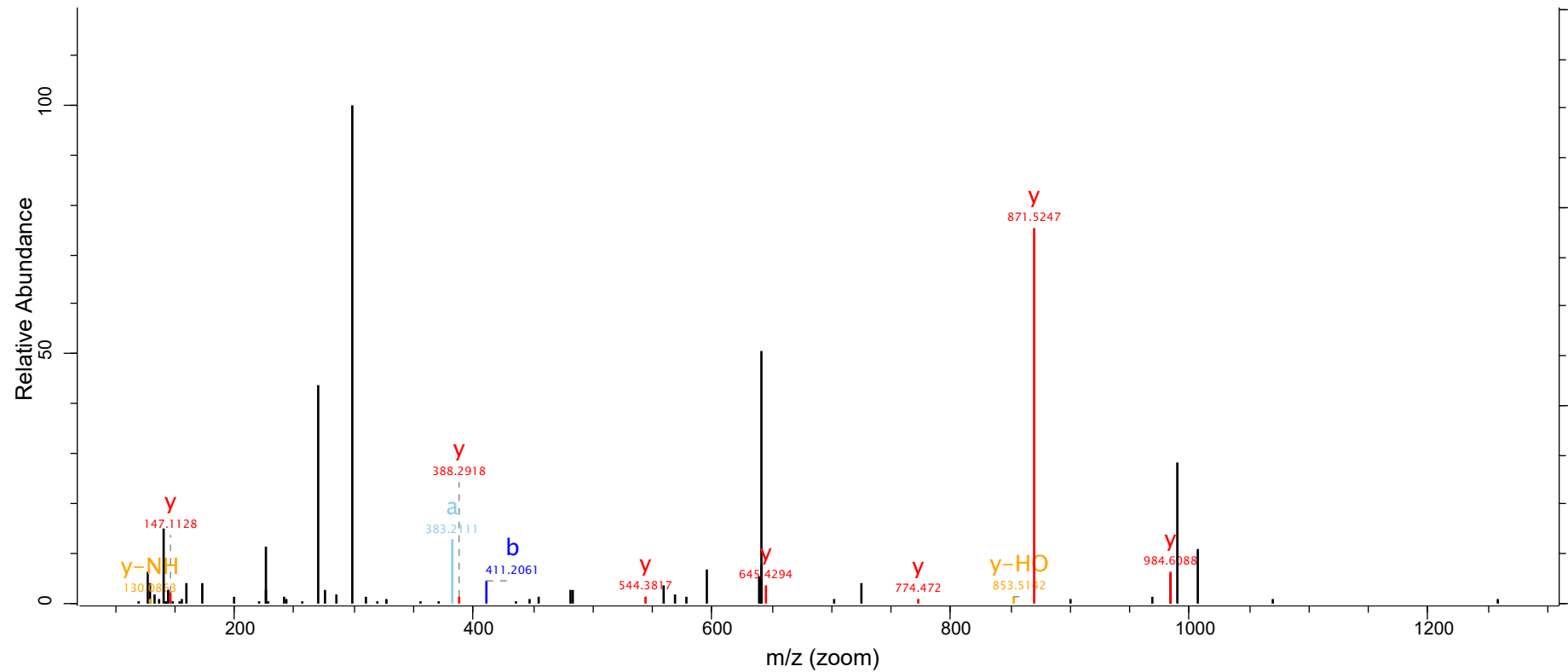
Scan
16908

Method
FTMS; HCD

Score
57.96

m/z
641.36

Gene names
TPST1



Raw File
agg_pep_sort_pos_1

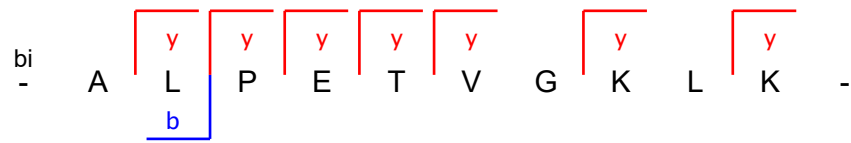
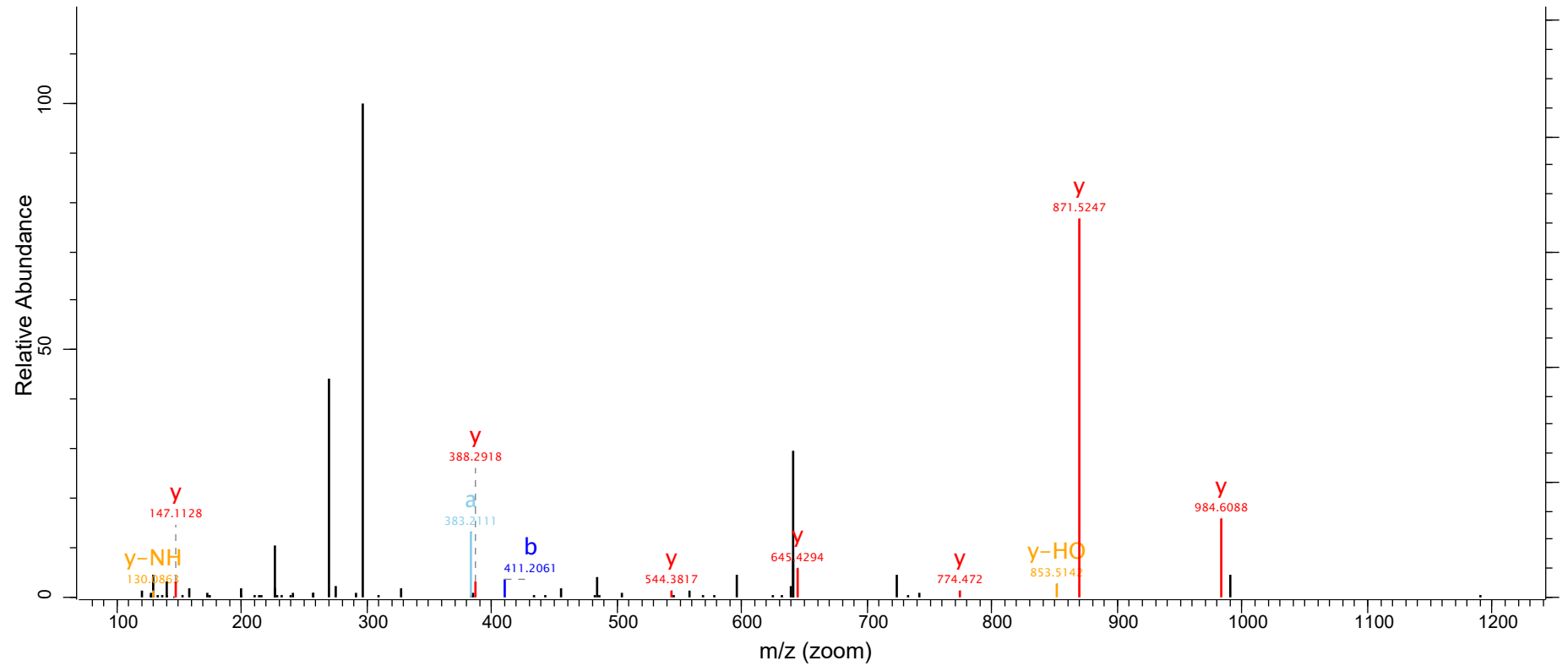
Scan
17295

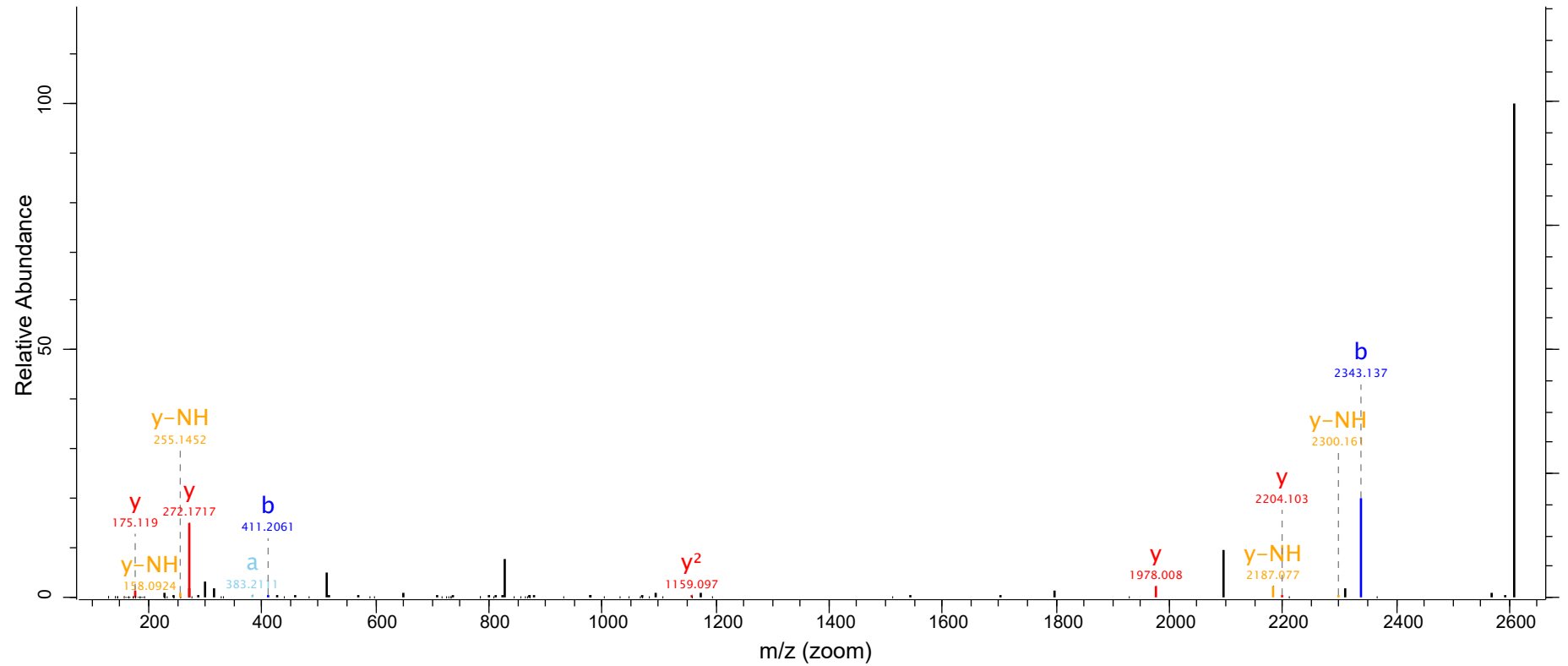
Method
FTMS; HCD

Score
69.42

m/z
641.37

Gene names
TPST1





Raw File
agg_pep_sort_pos_1

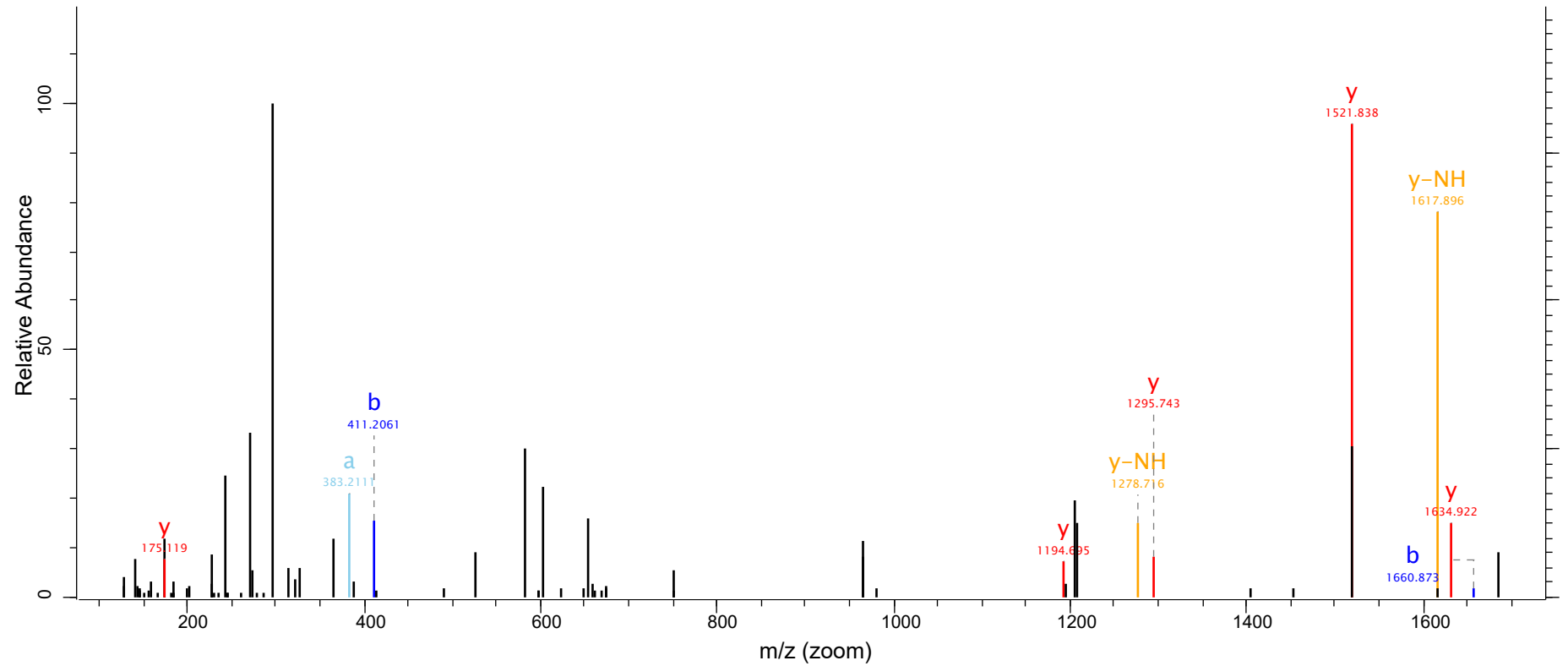
Scan
16987

Method
FTMS; HCD

Score
57.35

m/z
967.02

Gene names
WASF2



Raw File
agg_pep_sort_pos_1

Scan
12281

Method
FTMS; HCD

Score
57.8

m/z
524.6

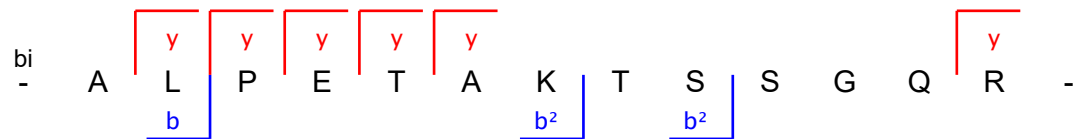
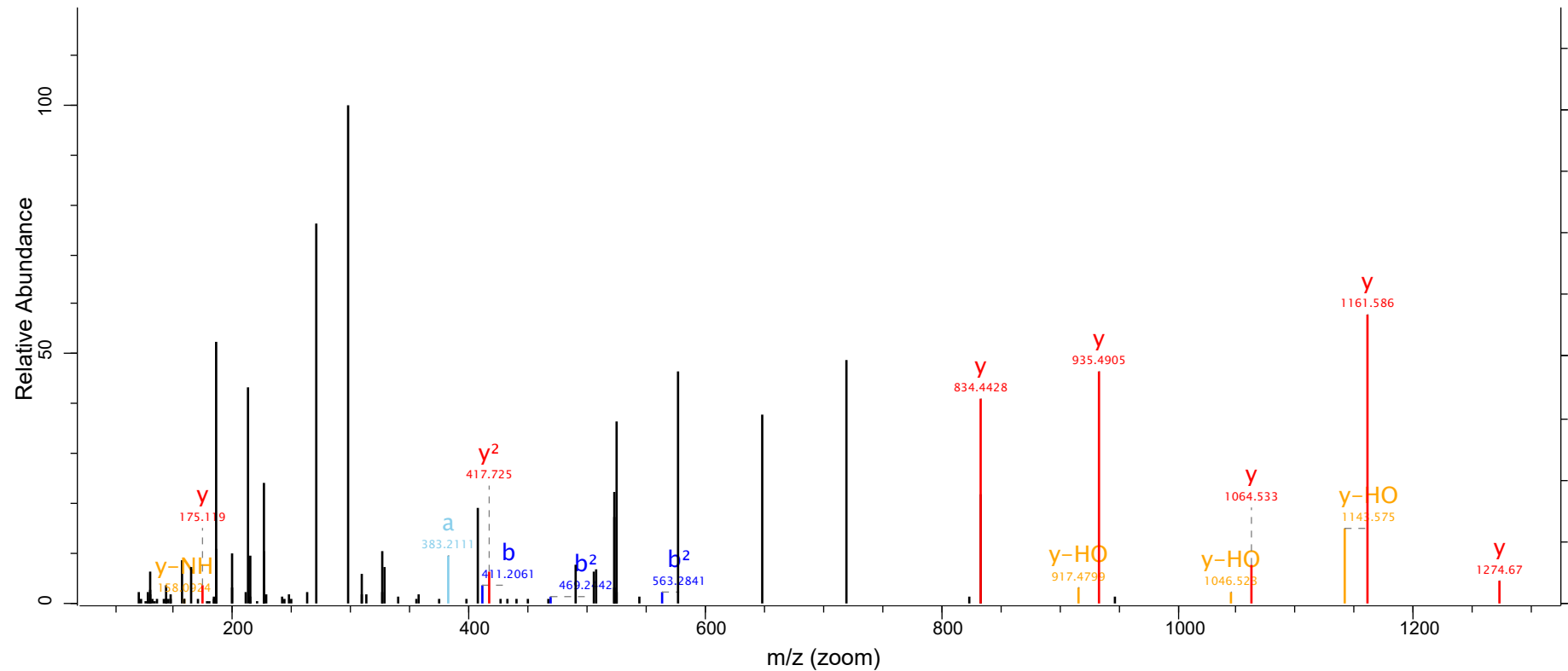


Figure S3. MS/MS spectra of Biotin-ALPET modified peptides (confident assignments) detected using chemoproteomic approach presented in Fig. 3. MS/MS spectra for a given peptide were selected based on the highest Andromeda score. All spectra contain a singly charged diagnostic ion of $m/z = 227.0849$ that represents the biotin-originated b-type ion. Spectra have been arranged alphabetically according to the gene names returned from the database search.

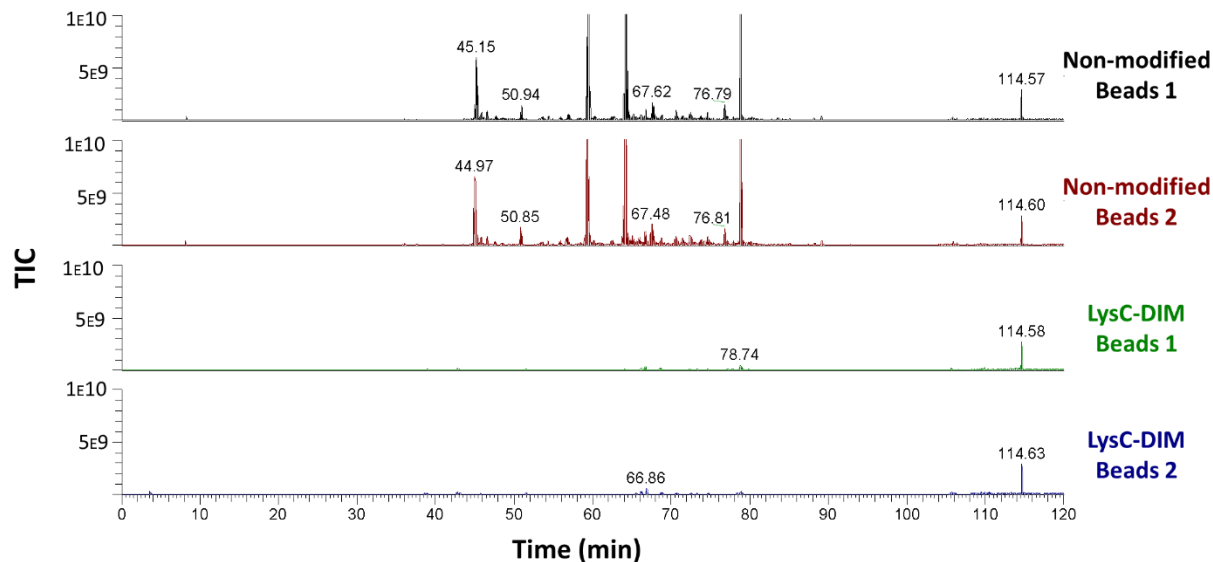


Figure S4. Capping NeutrAvidin lysines by dimethylation followed by a two-step on-bead LysC/off-bead trypsin digestion significantly reduces NeutrAvidin-derived high intensity peaks. Samples containing 200 μg of Biotin-ALPET labeled proteins, originated from MDA-MB-231 cells, were incubated in duplicate with 200 μL of dimethylated NeutrAvidin agarose beads or with 200 μL of non-derivatized NeutrAvidin agarose beads for 2 h at RT. Samples incubated with dimethylated beads were subjected to 1 h LysC digestion followed by digestion off-bead with trypsin overnight. Samples incubated with non-derivatized beads were subjected to an overnight on-bead digestion with trypsin. The plots show total ion current (TIC) chromatograms recorded for each of the four samples.

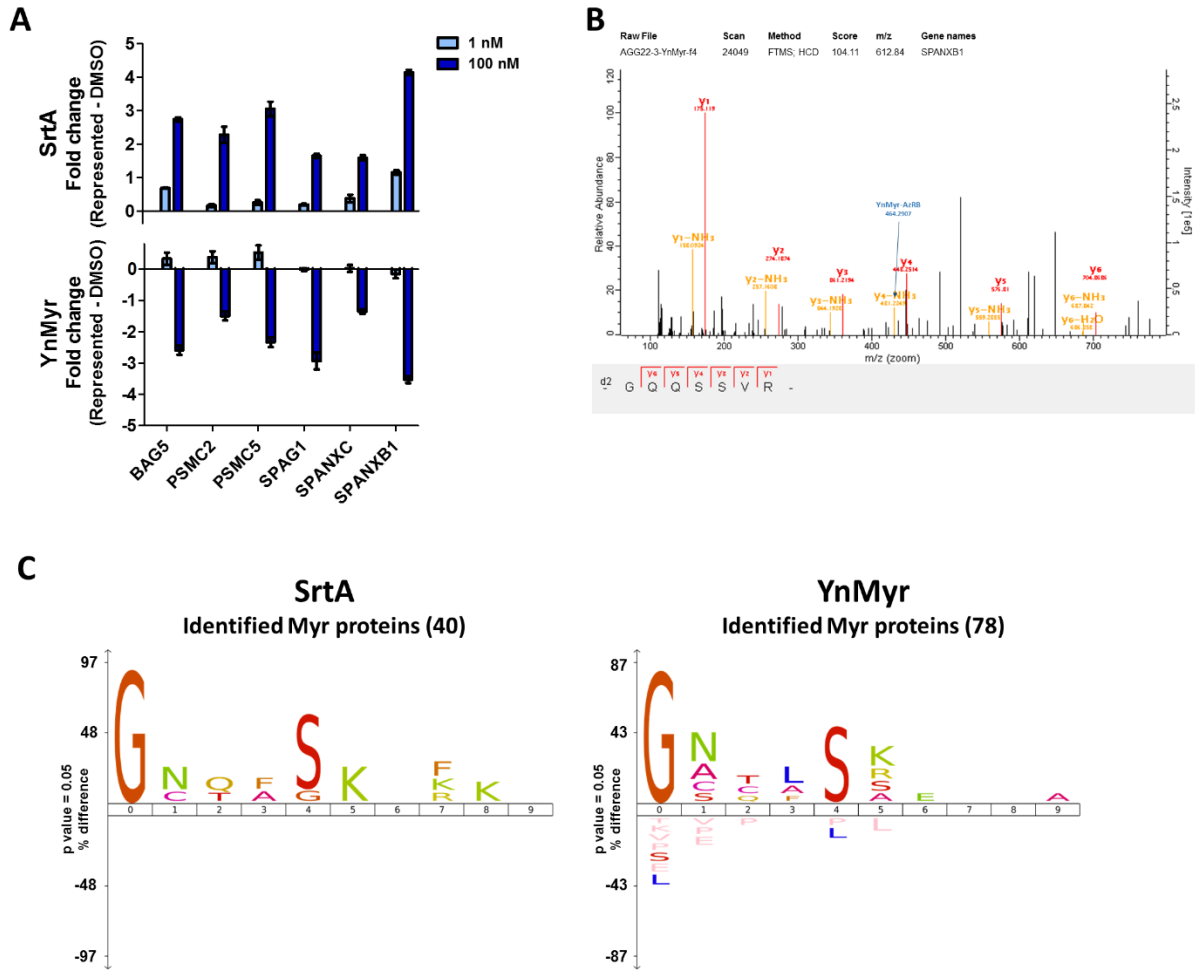


Figure S5. Comparison of SrtA-labeling with YnMyr metabolic tagging to assess NMT activity by protein-enrichment proteomics analysis. (A) Enrichment-derived intensities of significant proteins not previously known to be myristoylated. **(B)** Annotated MS/MS spectrum of SPANXB1 N-terminal peptide showing modification with YnMyr-AzRB. **(C)** Sequence logos of all known myristoylated proteins identified in SrtA and YnMyr protein-based enrichment experiments. Logos were generated with iceLogo (23), using the pre-compiled Swissprot human proteome (accessed on 17/09/2018) as the reference set.

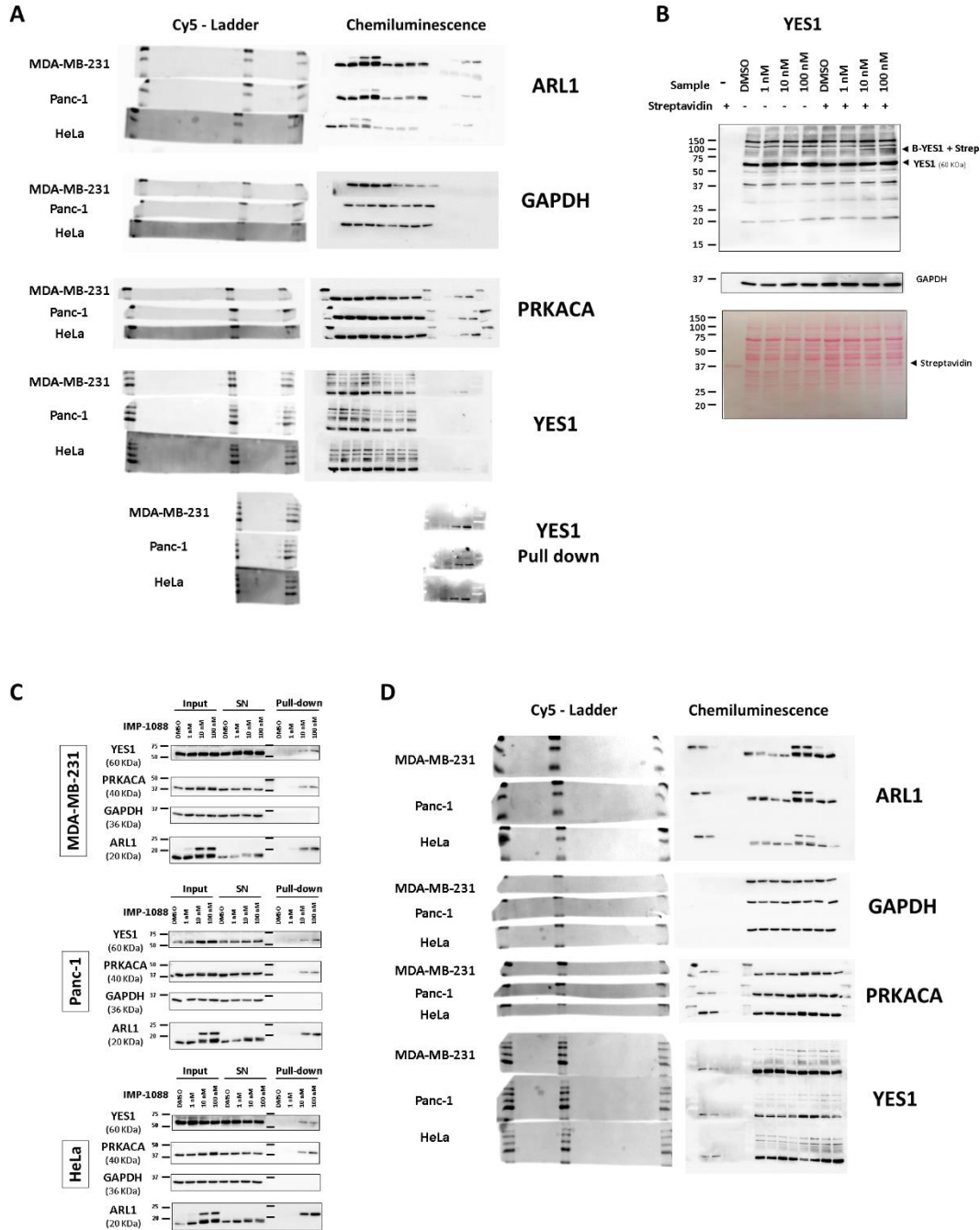


Figure S6. (A) Full membrane view of the western blots shown in Fig. 5A. (B) Streptavidin shift assay followed by western blot analysis against YES1. (C) A second biological replicate of the pull-down western blot analysis of selected myristoylated proteins showing NMTi dose-dependent increase in enrichment for all proteins but loading control (GAPDH). (D) Full membrane view of western blots shown in (C).