Quantitative proteomics and cytology of rice pollen sterol-rich membrane domains reveals pre-established cell polarity cues in mature pollen

Bing Han [1, 3 §#], Ning Yang [1, 3 §], Hai Pu[2], Tai Wang[1, 3 *]

[1]Key Laboratory of Plant Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

[2]Bruker Daltonics Inc. (China), Beijing 100081, China

[3]Universityof Chinese Academy of Sciences, Beijing 100049, China

[§]These authors contributed equally to this work

[#]Present address: Department of Molecular Physiology and Biophysics, Vanderbilt University, USA

Email addresses for authors:

Bing Han: bing.han@Vanderbilt.Edu

Ning Yang: yangning07@mails.ucas.ac.cn

Hai Pu: puhai@tiandimed.com

Corresponding author:

Tai Wang, twang@ibcas.ac.cn; 0086-10-62836210

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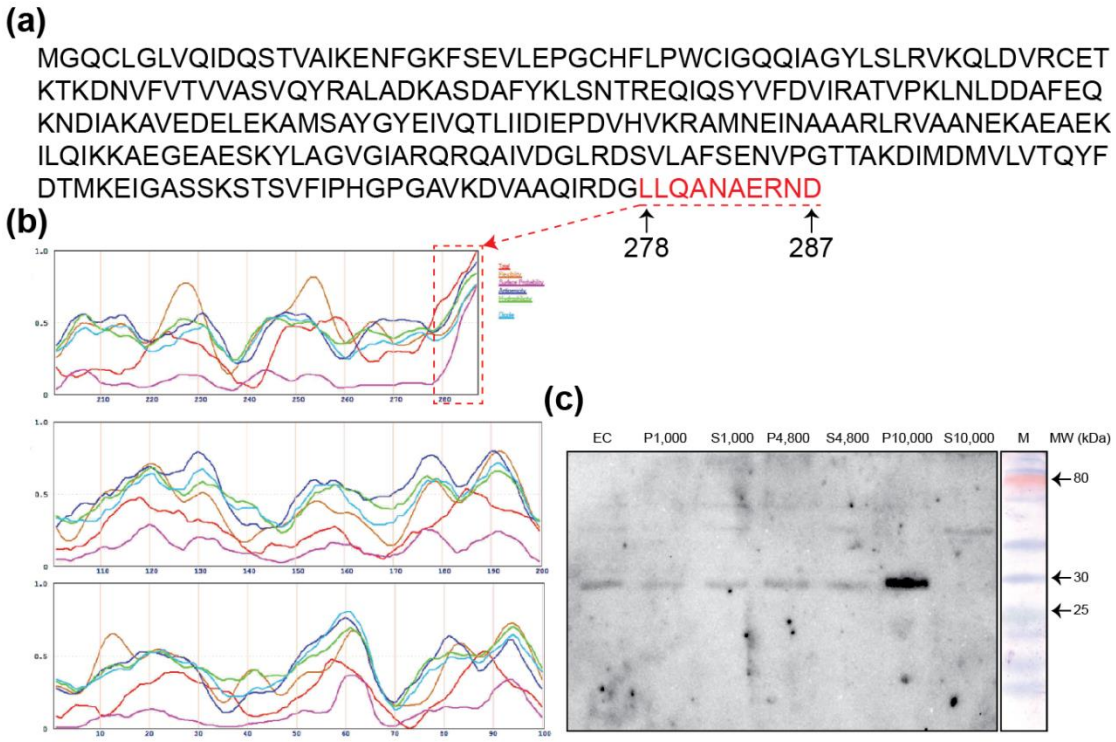


Figure S1. Flotillin-like protein antibody preparation. (a) Sequence of the flotillin-like protein (band_7 protein, BAD23328) and synthetic antigen. **(b)** Antigen prediction results (the software 'Epitope Adviser Lite' was developed by MBL, Woburn, MA, USA). **(c)** Antibody specificity testing in differential centrifugation products from rice pollen lysate. EC, entire cell lysate; P, pellet; S, supernatant; M, protein molecular weight marker.

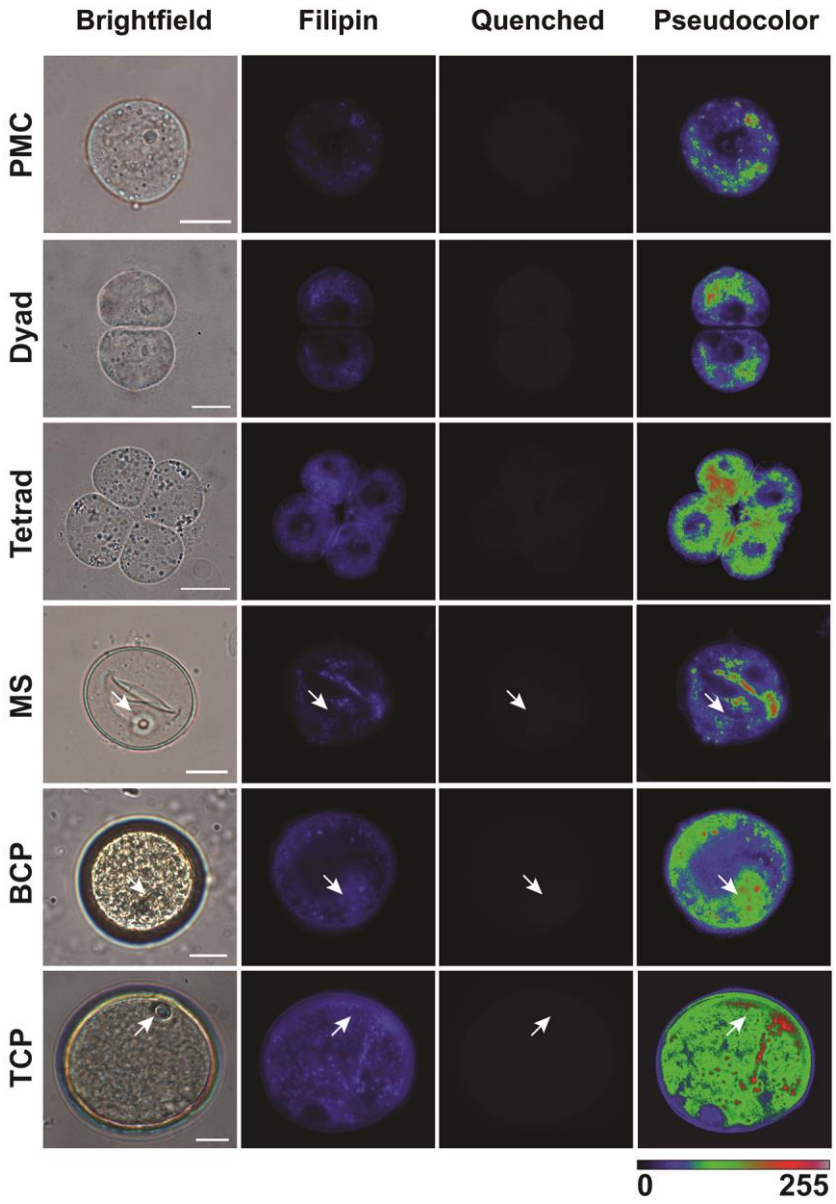


Figure S2. Distribution of sterols in developing pollen. Sterols were labeled with the fluorescent molecule, filipin. Pseudocolor images indicating the intensity of filipin-sterol fluorescence. Color bar represents the relative intensities from 0 to 255. PMC, pollen mother cell; MS, microspore; BCP, bicellular pollen; TCP, immature tricellular pollen. Arrow: aperture. Arrow head: pollen tube. Bars = 10μm.

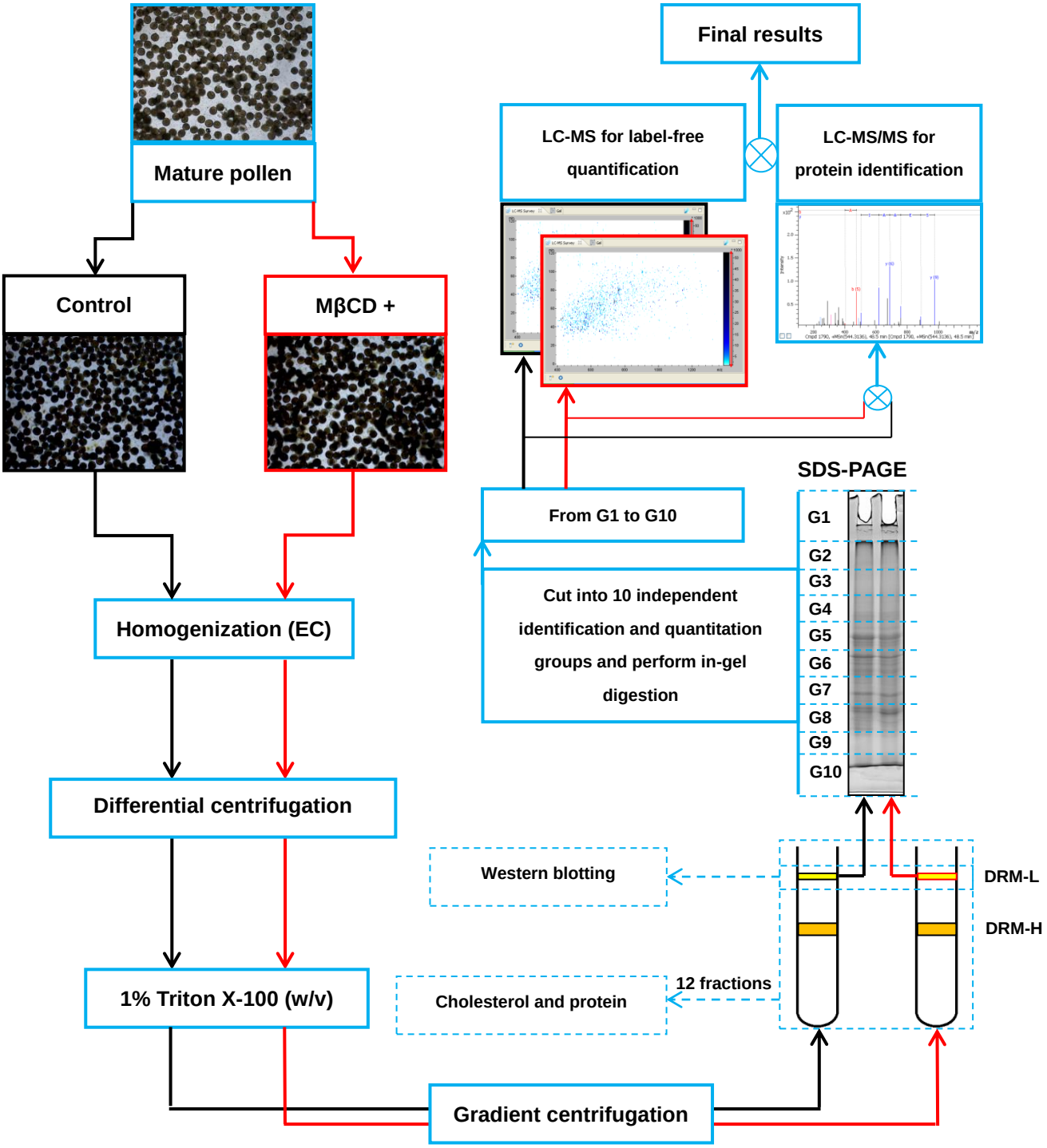


Figure S3. Work flow used to identify sterol-rich membrane microdomain-associated proteins of mature rice pollen. Five replicates were carried out in parallel. EC, entire cell lysate; DRM-L, detergent insoluble membrane-low density fraction; DRM-H, detergent insoluble membrane-high density fraction. Gn (n=1-10), group number.

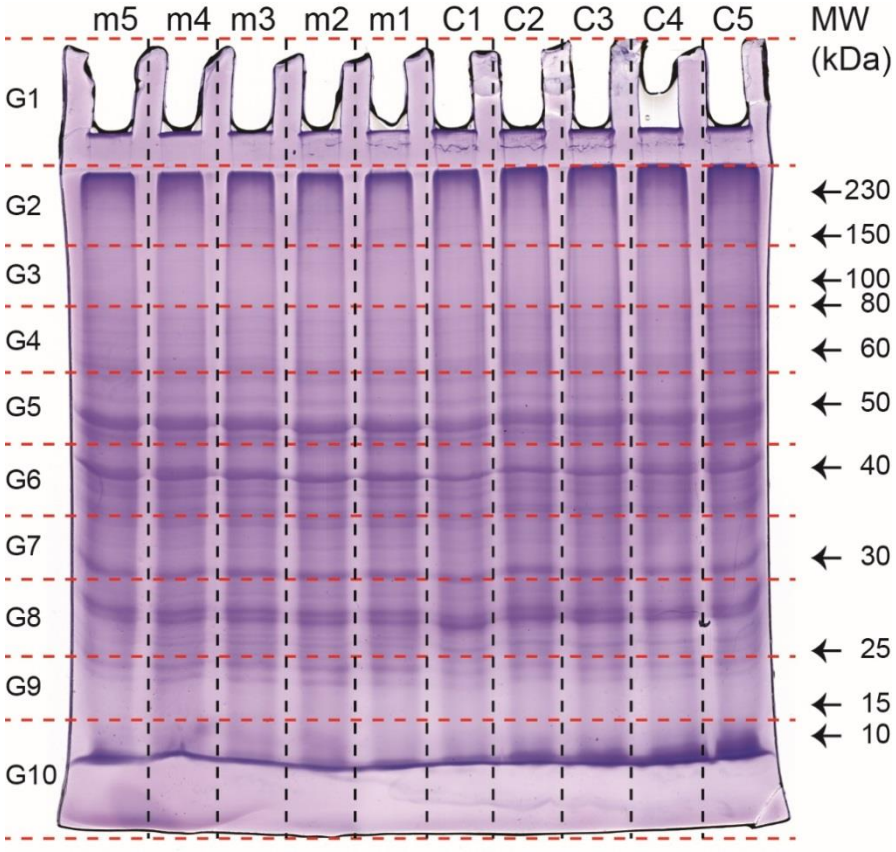


Figure S4. SDS-PAGE separation of proteins from rice pollen DRM-Ls. Proteins were resolved on 4.5% stacking gel and 12.5% separation gel with a gel thickness of 1mm. Proteins were stained in-gel with Coomassie blue. The whole gel was cut into 100 samples along the dotted line, then each sample was cut into 1 cubic millimeter (mm^3) pieces for in-gel tryptic digestion. The 10 samples between the two red dotted lines belong to 1 independent analysis group. Gn (n=1-10), group number; C, control; T, treatment; M, protein molecular weight marker.

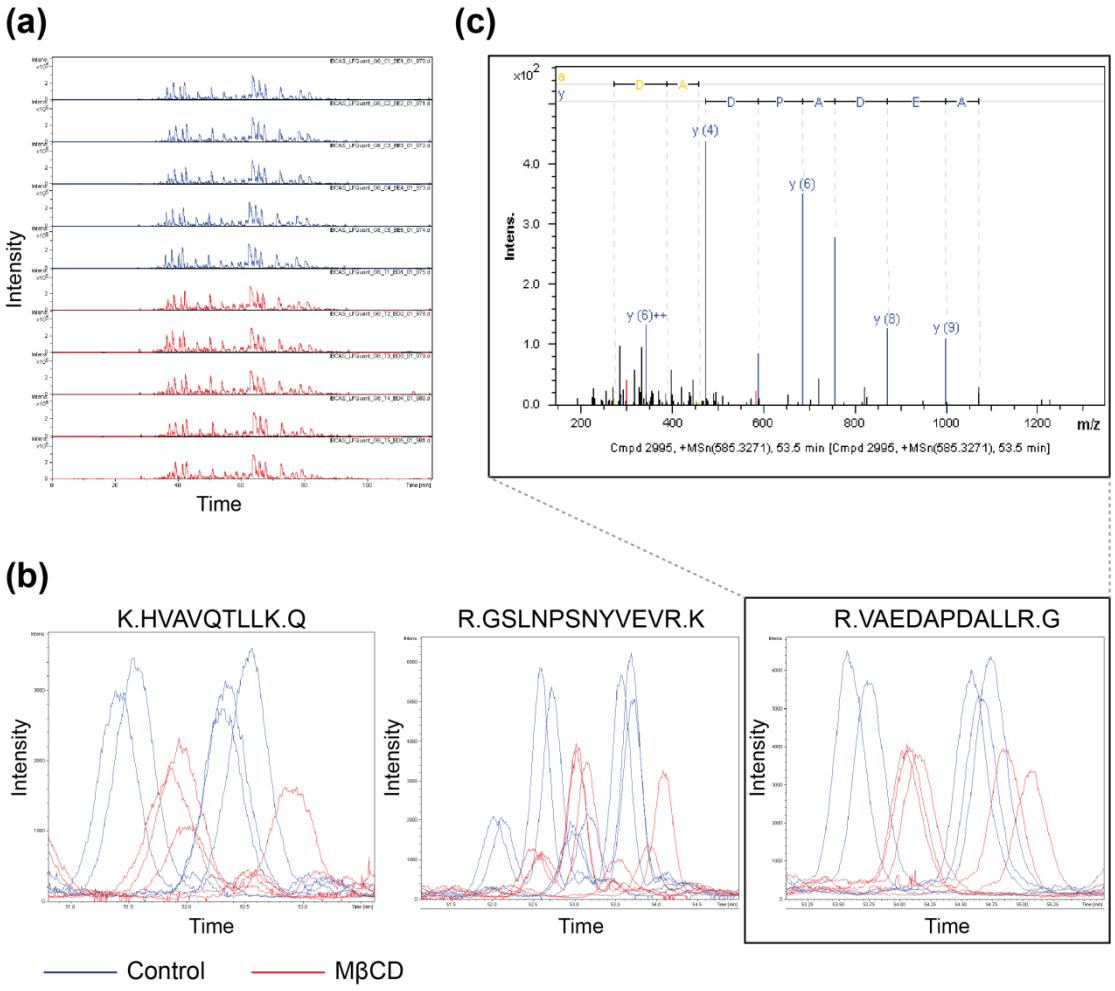
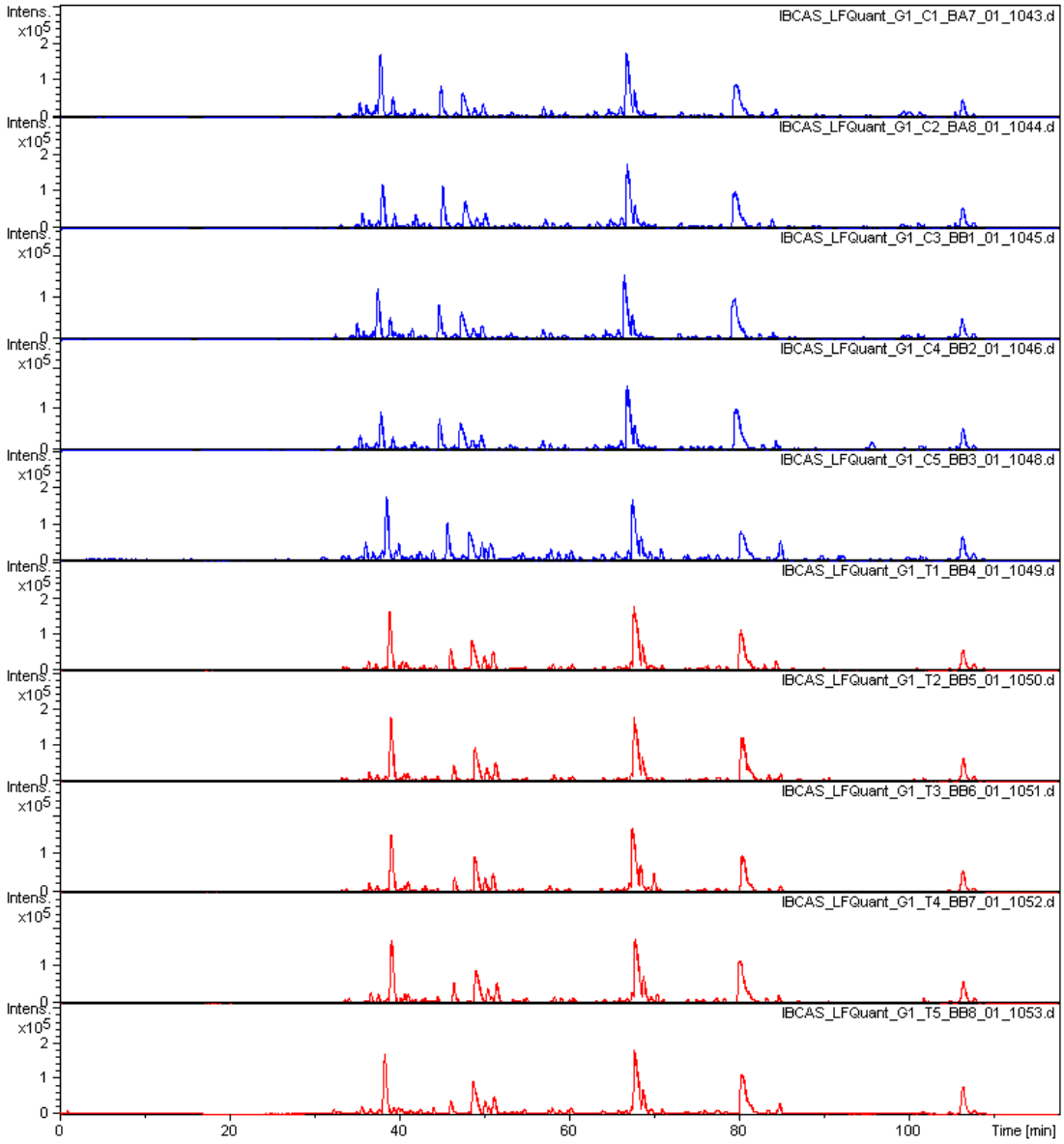


Figure S5. Representative images for protein identification and quantification with ESI-Qq-TOF mass spectrometer. **(a)** Basic Peak Chromatogram of group 6. **(b)** Extracted Ion Chromatogram of three peptides matched to gi|31126785. **(c)** A representative MS/MS fragmentation spectra. In **(a)** and **(b)**, the blue line indicates signals in control samples; the red line indicates signals of MβCD treated samples.

Group 1


Intens.
x10^5
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IBCAS_LFQuant_G1_C4_BB2_01_1046.d
IBCAS_LFQuant_G1_C5_BB3_01_1048.d
IBCAS_LFQuant_G1_T1_BB4_01_1049.d
IBCAS_LFQuant_G1_T2_BB5_01_1050.d
IBCAS_LFQuant_G1_T3_BB6_01_1051.d
IBCAS_LFQuant_G1_T4_BB7_01_1052.d
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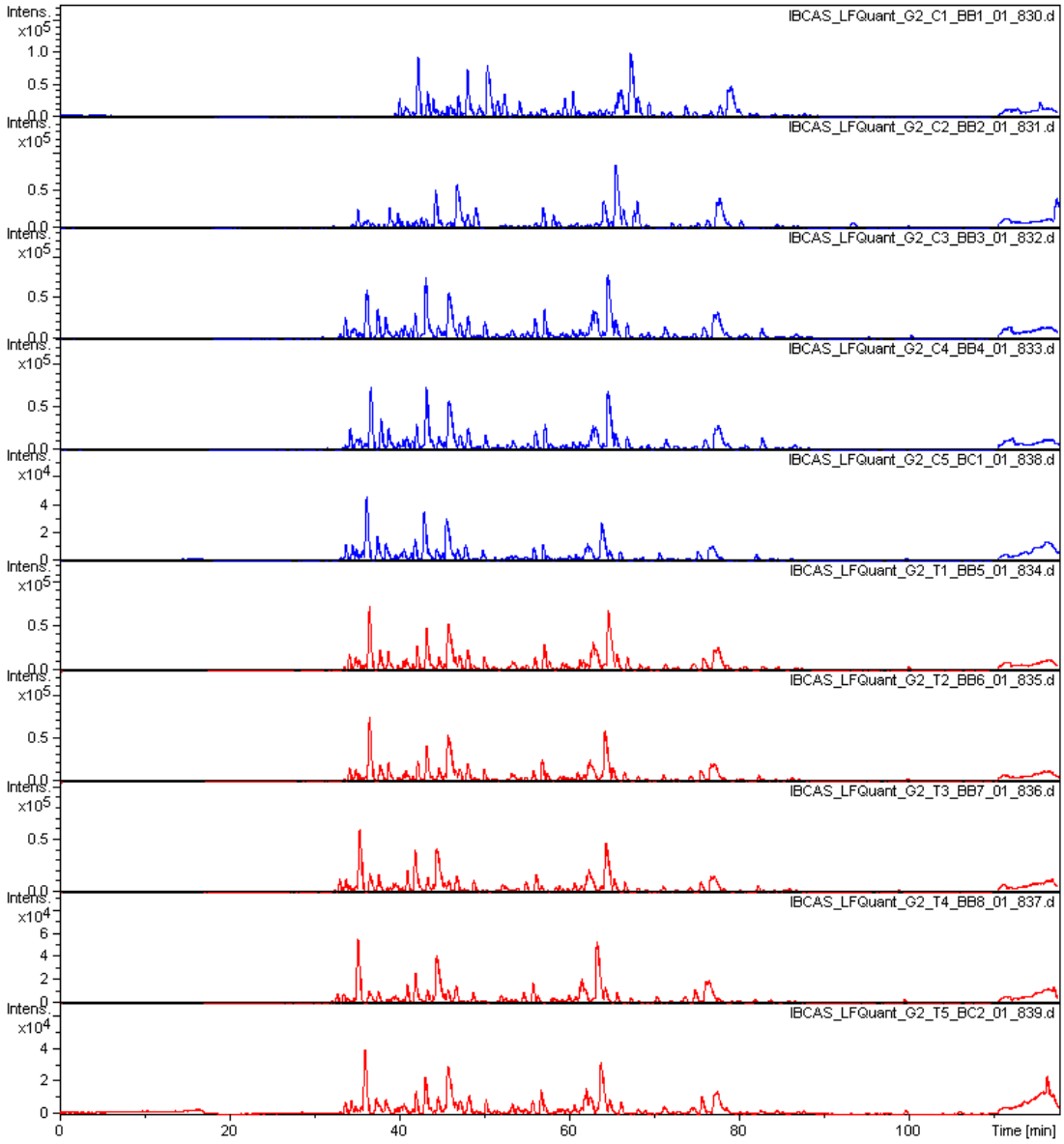
20
40
60
80
100
Time [min]

Group 2


Intens.
x10^5
1.0
0.5
0.0
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IBCAS_LFQuant_G2_C3_BB3_01_832.d
IBCAS_LFQuant_G2_C4_BB4_01_833.d
Intens.
x10^4
4
2
0
IBCAS_LFQuant_G2_C5_BC1_01_838.d
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IBCAS_LFQuant_G2_T2_BB6_01_835.d
IBCAS_LFQuant_G2_T3_BB7_01_836.d
6
IBCAS_LFQuant_G2_T4_BB8_01_837.d
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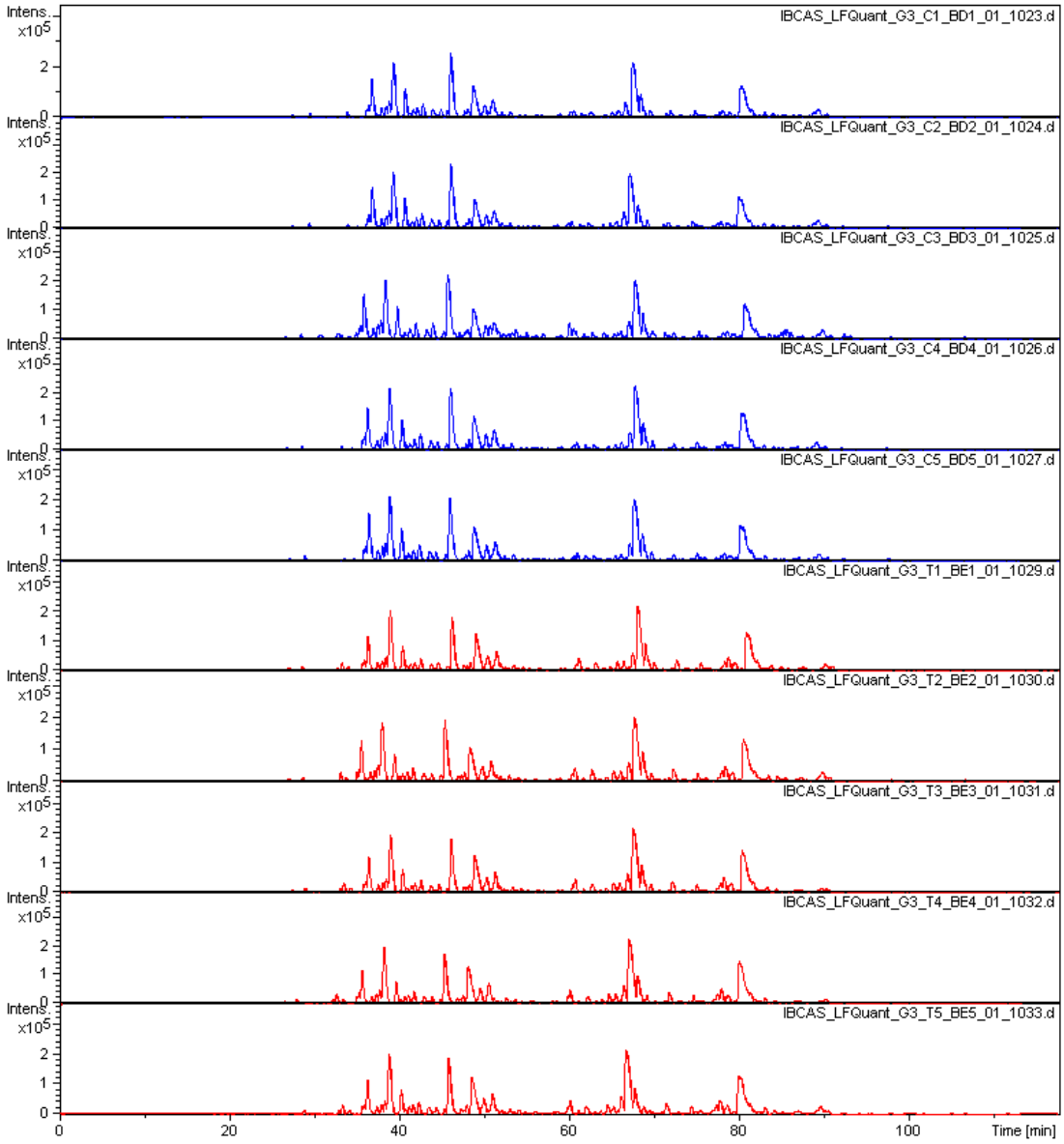
20
40
60
80
100
Time [min]

Group 3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x10^5
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IBCAS_LFQuant_G3_C2_BD2_01_1024.d
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IBCAS_LFQuant_G3_C4_BD4_01_1026.d
IBCAS_LFQuant_G3_C5_BD5_01_1027.d
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IBCAS_LFQuant_G3_T4_BE4_01_1032.d
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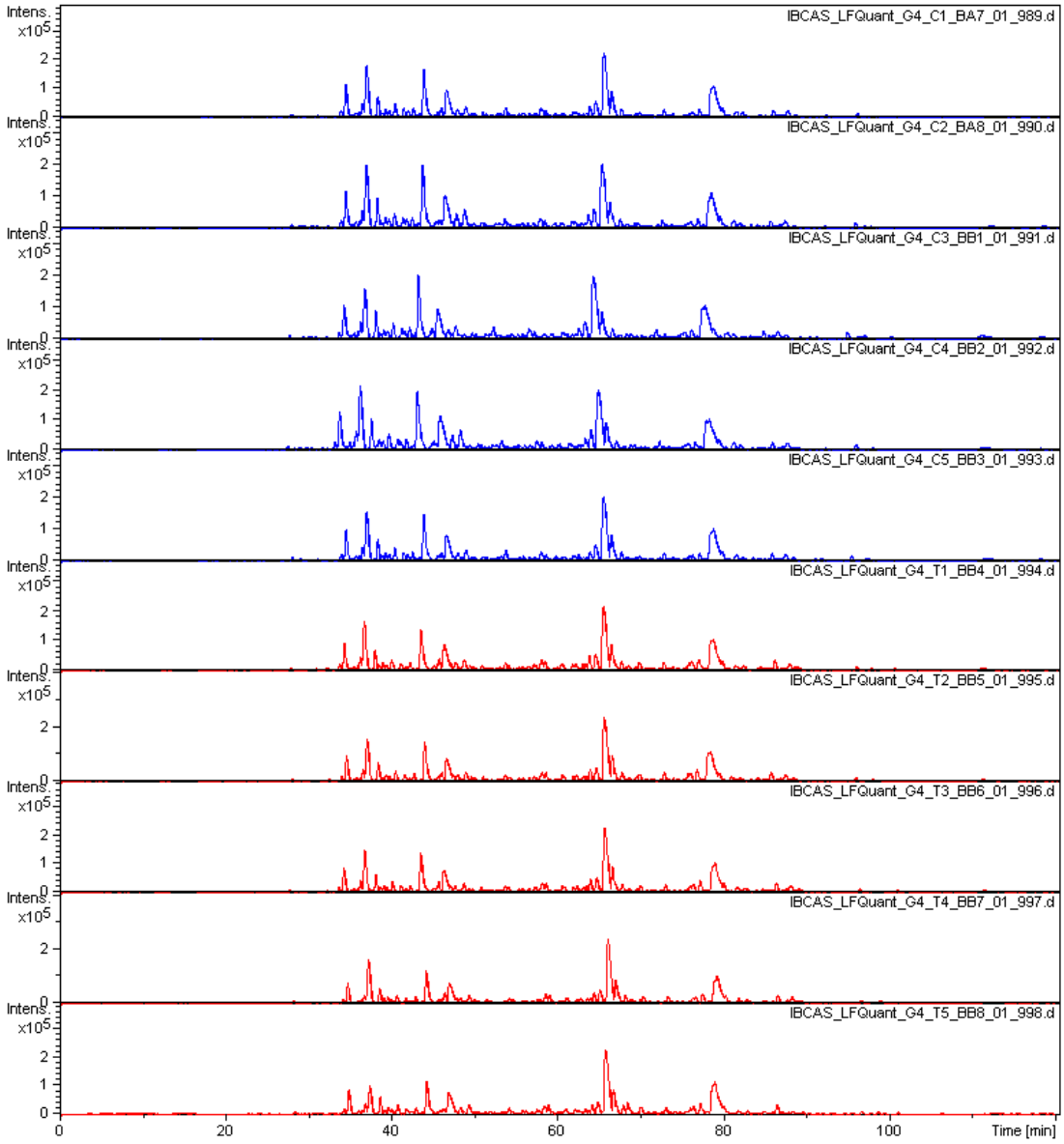
40
60
80
100
Time [min]

Group 4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x10^5
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IBCAS_LFQuant_G4_C4_BB2_01_992.d
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IBCAS_LFQuant_G4_T1_BB4_01_994.d
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IBCAS_LFQuant_G4_T3_BB6_01_996.d
IBCAS_LFQuant_G4_T4_BB7_01_997.d
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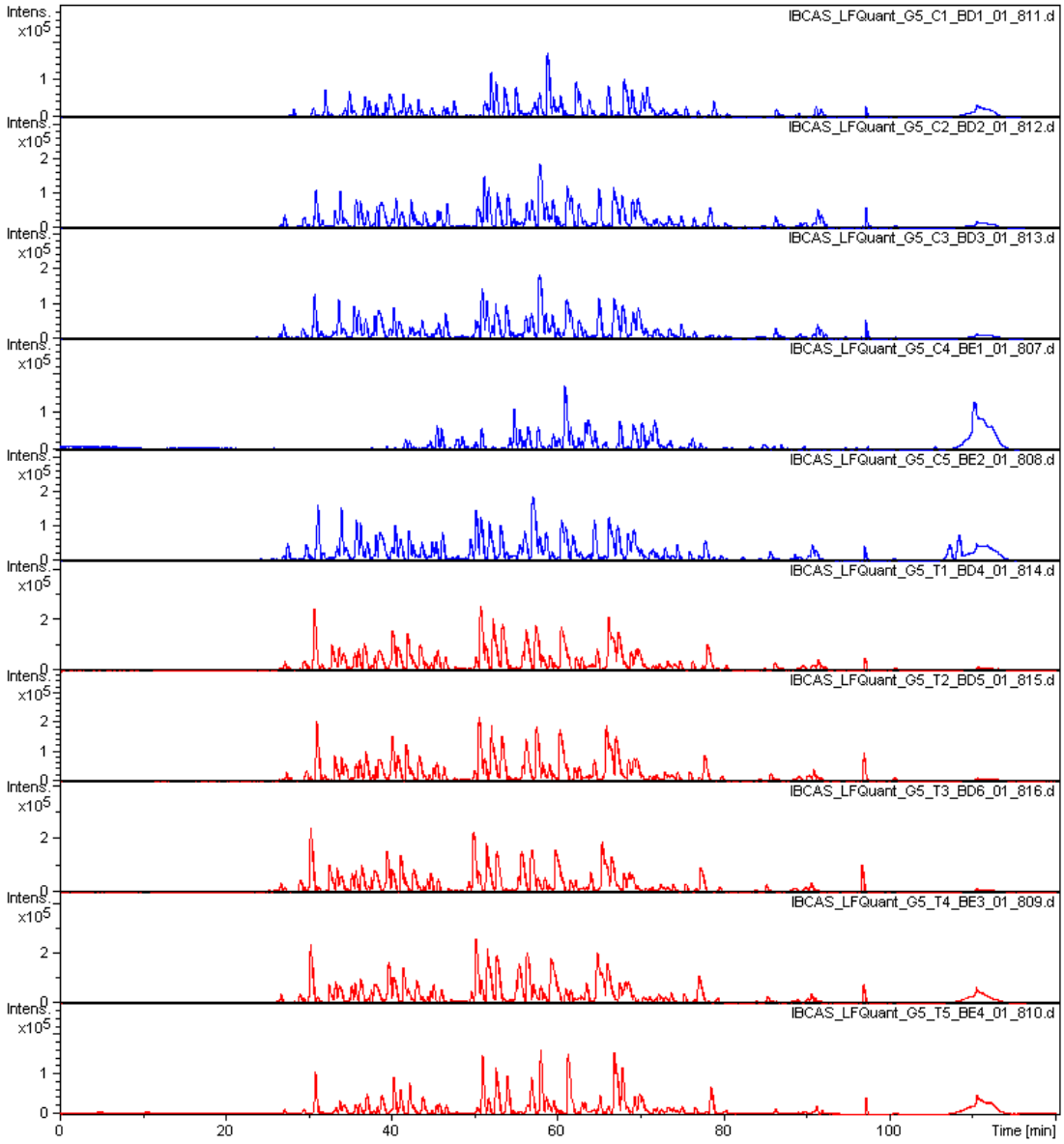
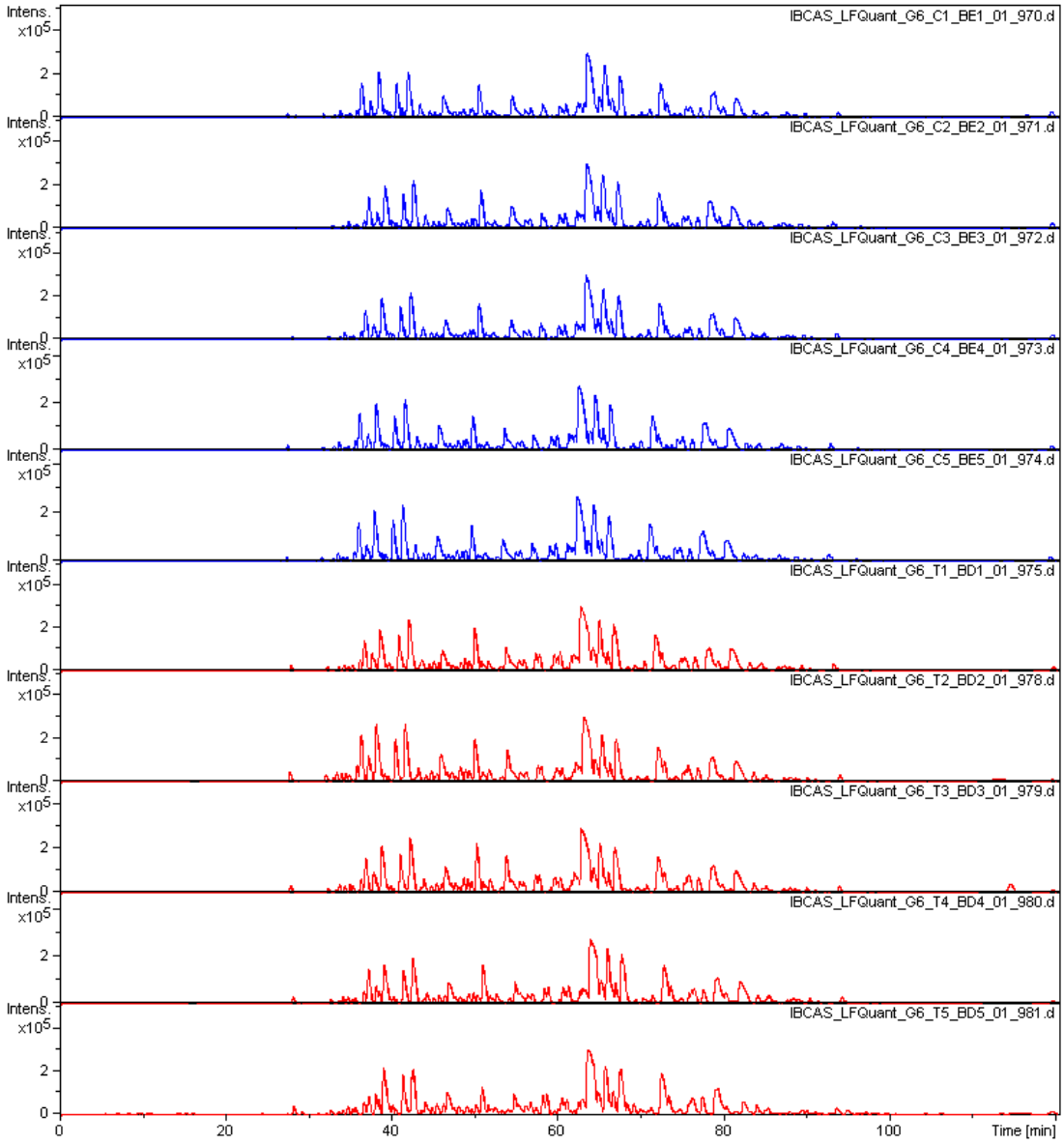
20
40
60
80
100
Time [min]

Group 5



The control sample of replicate #4 shows an approximate 20 minute delay, so this replicate was excluded from quantitative analysis.

Group 6


Intens.
x10^5
2
0
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IBCAS_LFQuant_G6_C4_BE4_01_973.d
IBCAS_LFQuant_G6_C5_BE5_01_974.d
IBCAS_LFQuant_G6_T1_BD1_01_975.d
IBCAS_LFQuant_G6_T2_BD2_01_978.d
IBCAS_LFQuant_G6_T3_BD3_01_979.d
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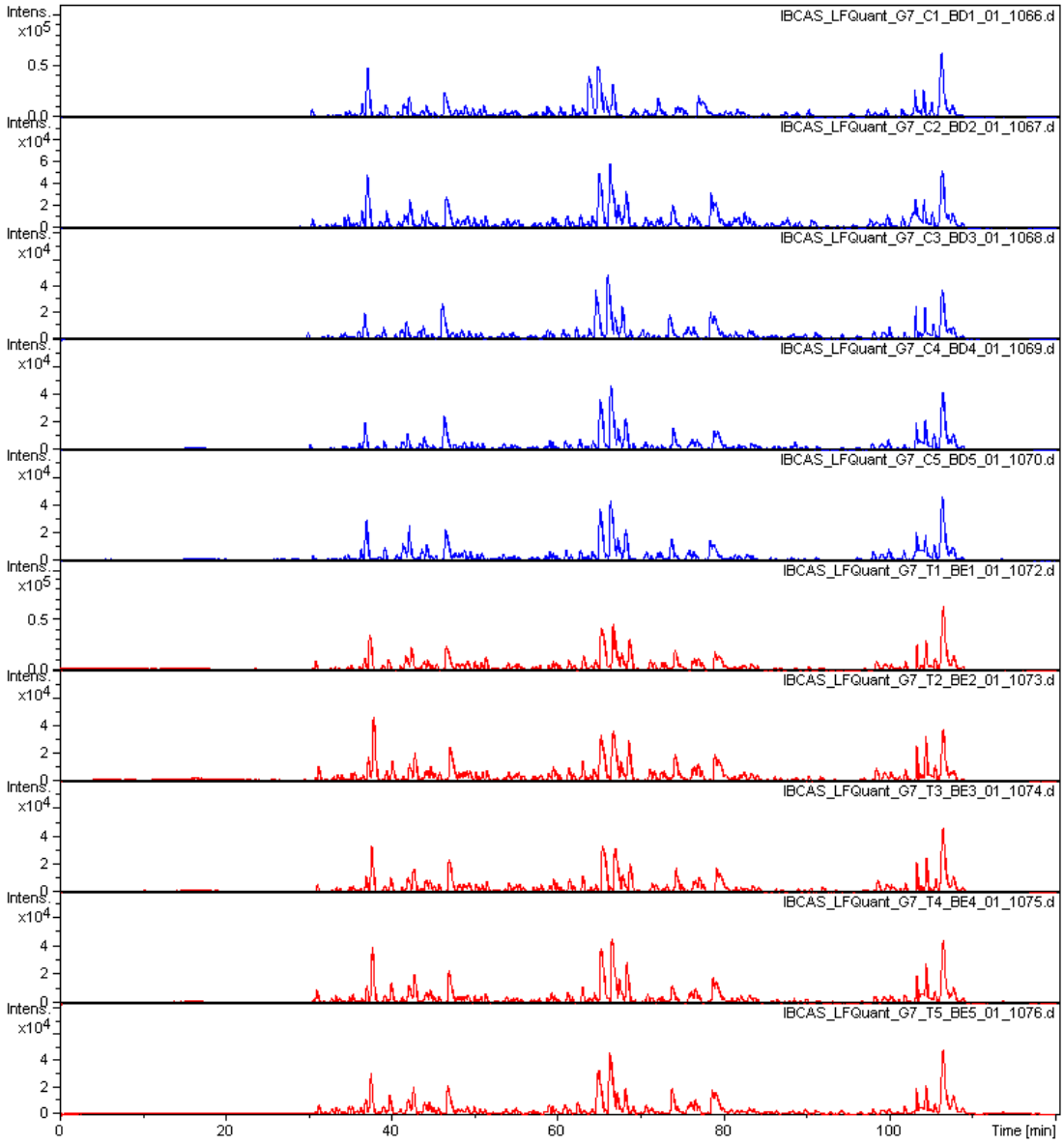
20
40
60
80
100
Time [min]

Group 7


Intens.
x10^5
0.5
0.0
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Intens.
x10^4
6
4
2
0
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Intens.
x10^4
4
2
0
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Intens.
x10^4
4
2
0
IBCAS_LFQuant_G7_C4_BD4_01_1069.d
Intens.
x10^4
4
2
0
IBCAS_LFQuant_G7_C5_BD5_01_1070.d
Intens.
x10^5
0.5
0.0
IBCAS_LFQuant_G7_T1_BE1_01_1072.d
Intens.
x10^4
4
2
0
IBCAS_LFQuant_G7_T2_BE2_01_1073.d
Intens.
x10^4
4
2
0
IBCAS_LFQuant_G7_T3_BE3_01_1074.d
Intens.
x10^4
4
2
0
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Intens.
x10^4
4
2
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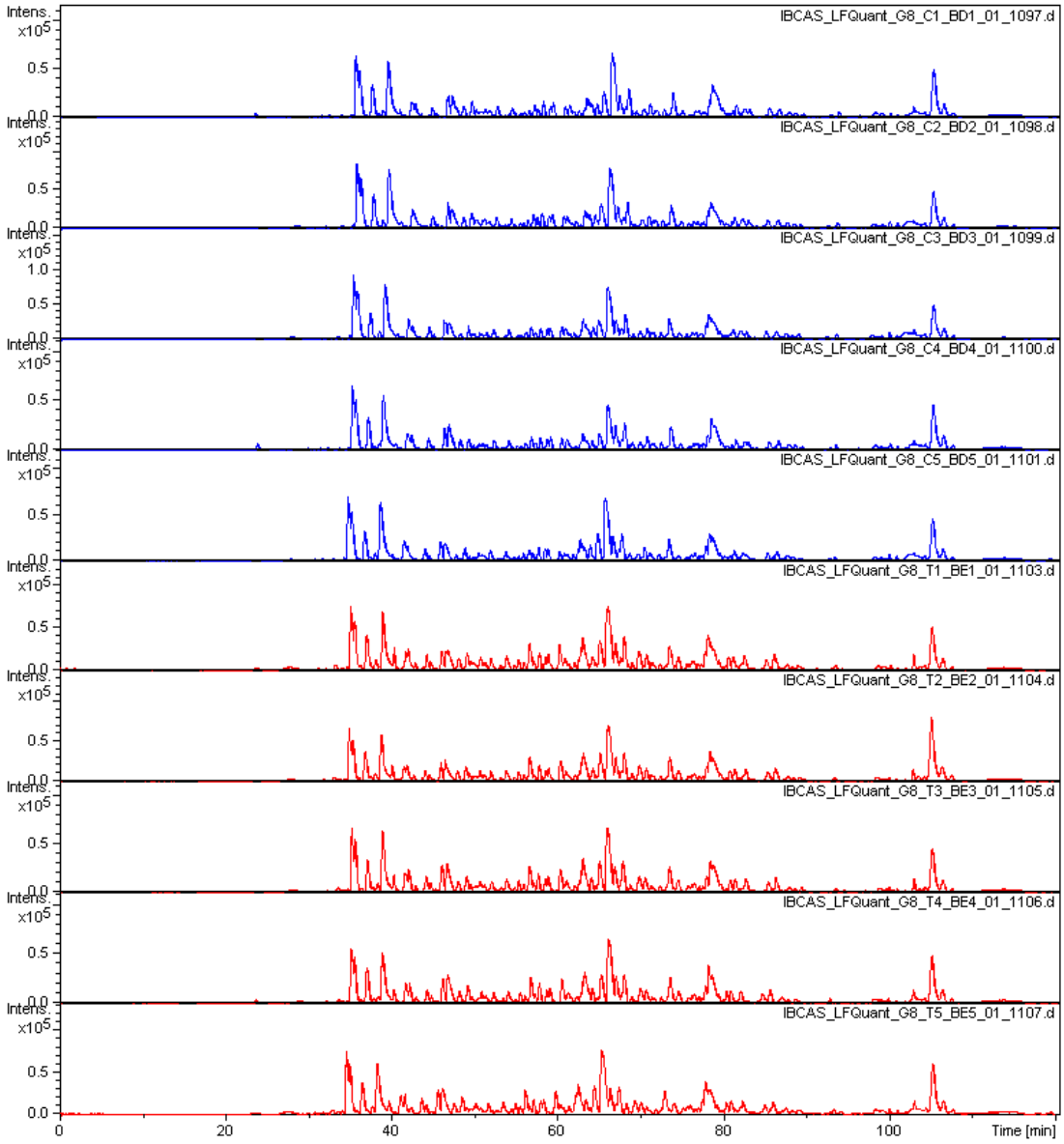
0
20
40
60
80
100
Time [min]

Group 8


Intens.
x10^5
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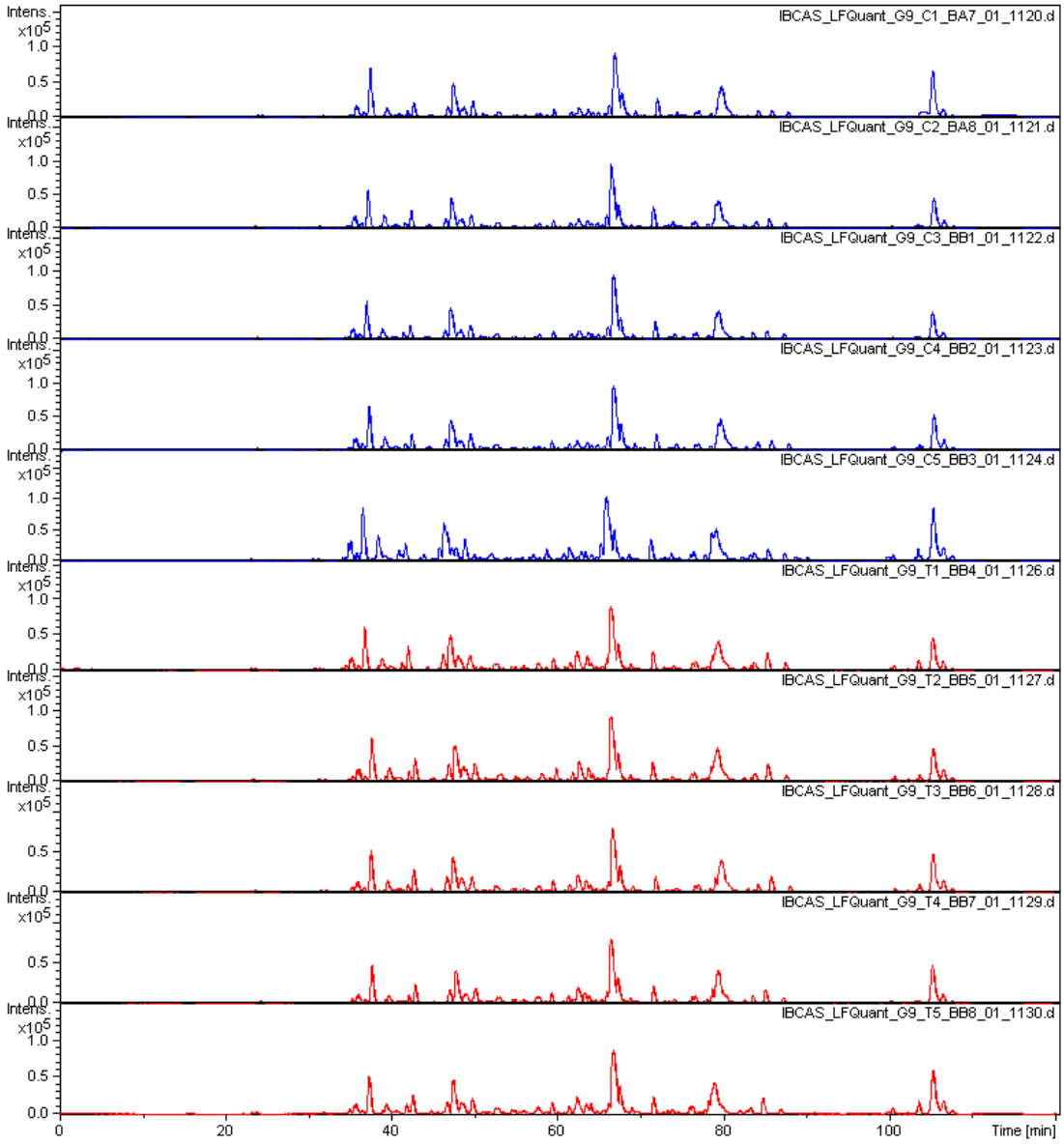
IBCAS_LFQuant_G8_T4_BE4_01_1106.d
IBCAS_LFQuant_G8_T5_BE5_01_1107.d
0.0
0.5
1.0
0
20
40
60
80
100
Time [min]

Group 9


Intens.
x10^5
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0.5
0.0
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80
100
Time [min]

Group 10

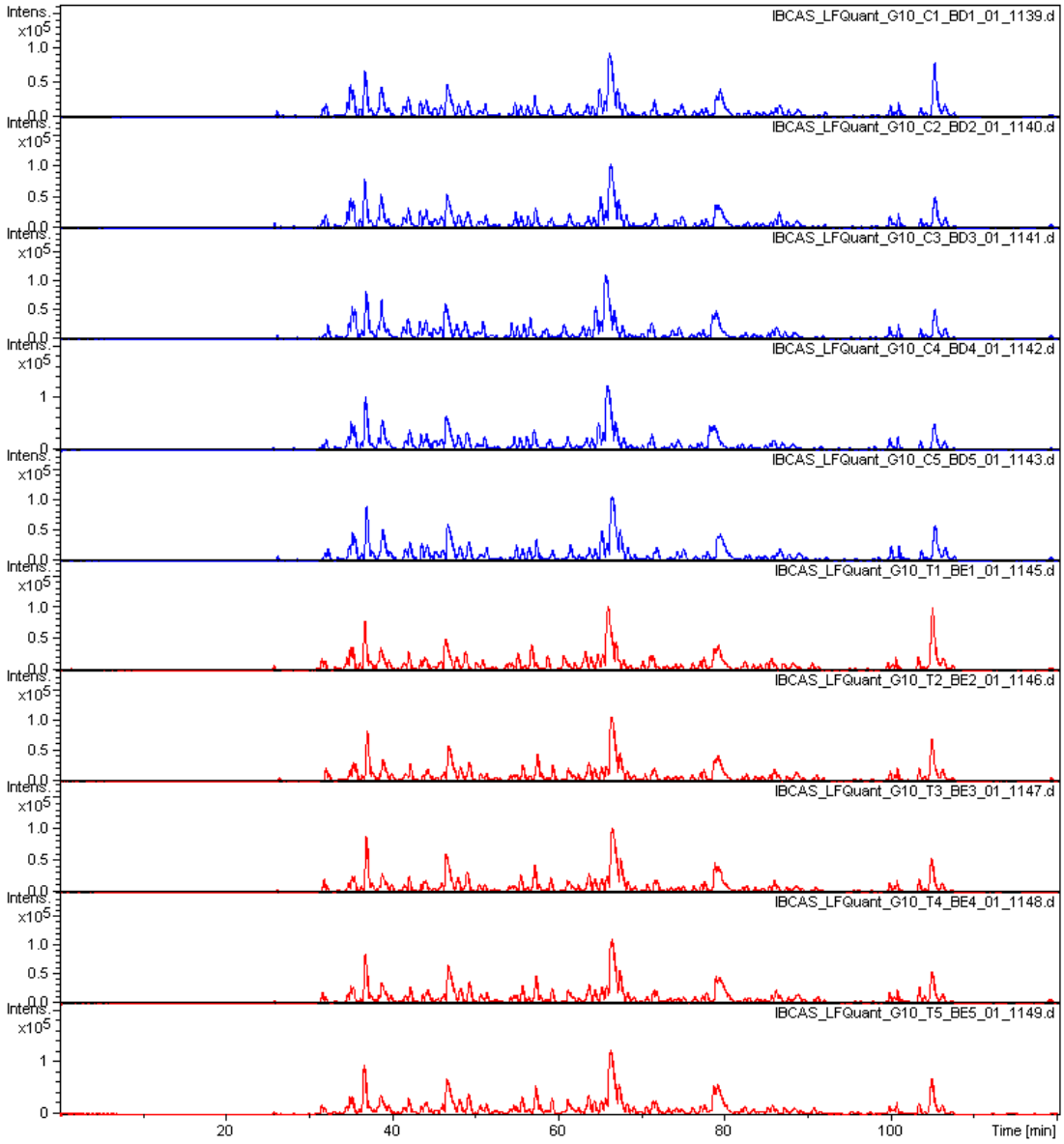
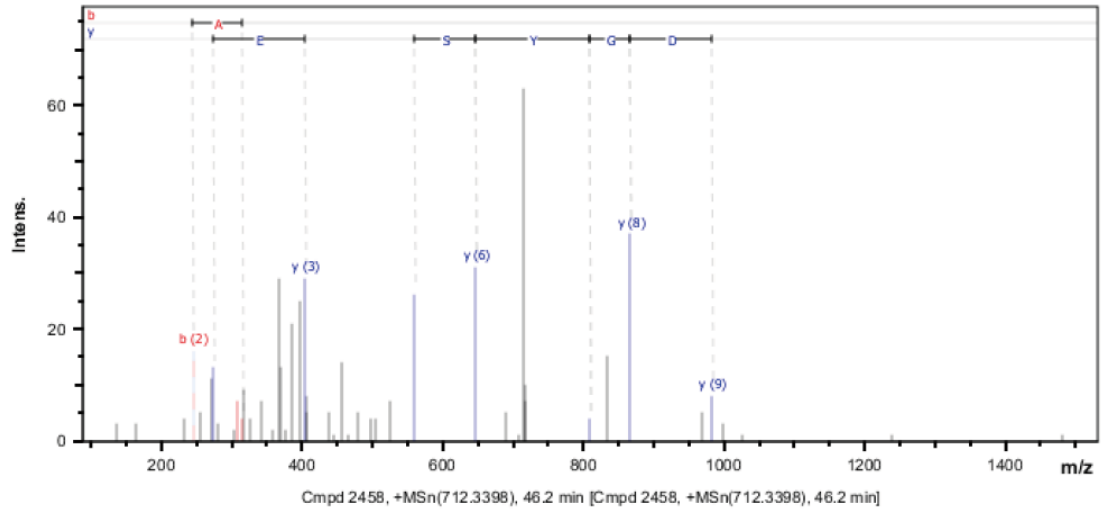
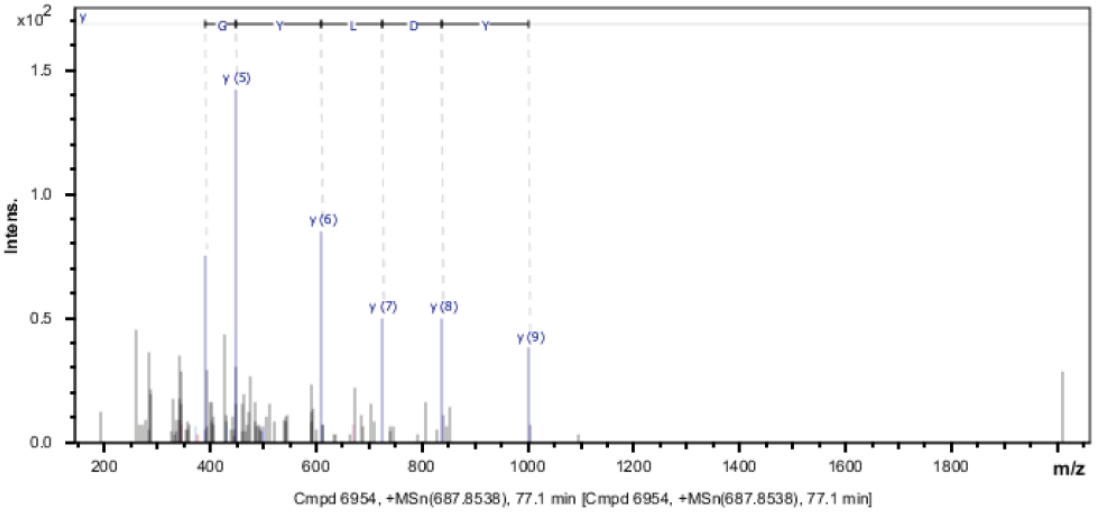


Figure S6. Comparisons of the chromatograph profiles among these duplicates (from analysis group 1 to 10).

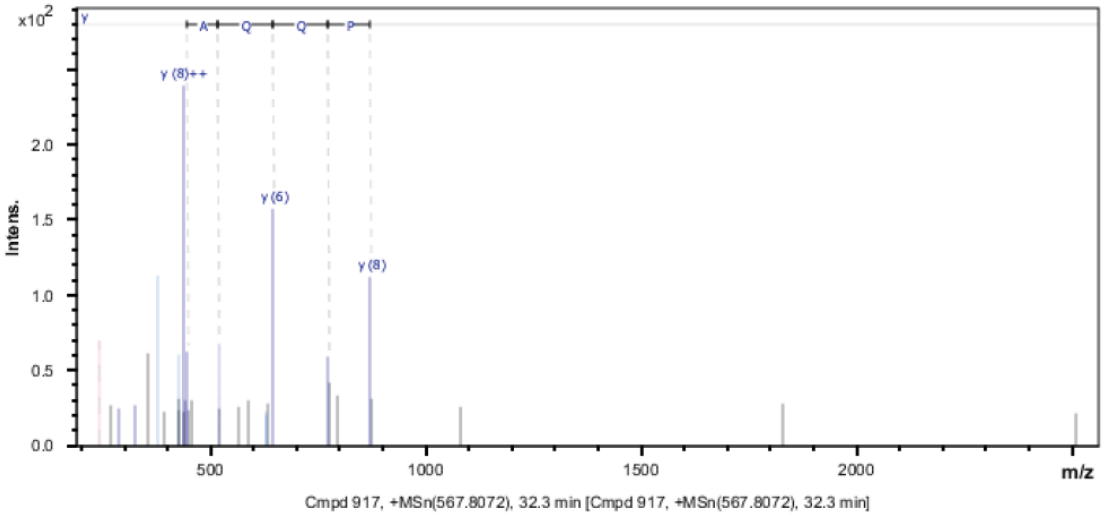
Protein Name: 40S ribosomal protein S3, putative, expressed [Oryza sativa (japonica cultivar-group)]
Accession: gi|108709466 **Parent m/z:** 712.335999, 2+
Sequence: R.ELAEDGYSGVEVR.V
Score: 40.91



Protein Name: Lysyl-tRNA synthetase, putative, expressed [Oryza sativa (japonica cultivar-group)]
Accession: gi|108709561 **Parent m/z:** 687.845642, 2+
Sequence: K.LLFYDLYGGGEK.V
Score: 43.89

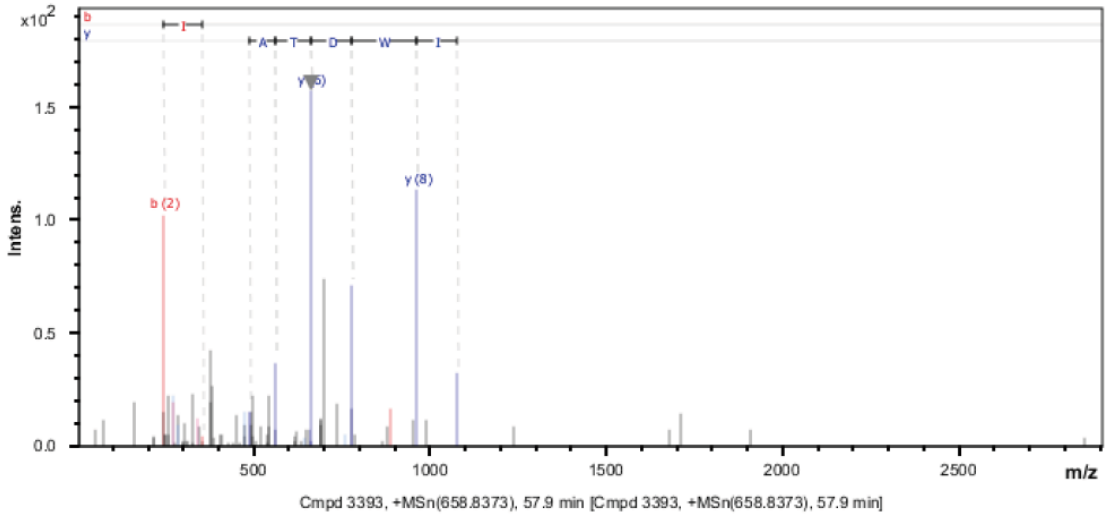


Protein Name: Eukaryotic translation initiation factor 5A-2, putative, expressed [Oryza sativa (japonica cultivar-group)]
Accession: gi|108711192 **Parent m/z:** 567.8072, 2+
Sequence: K.TYPQQAGTIR.K
Score: 34.18



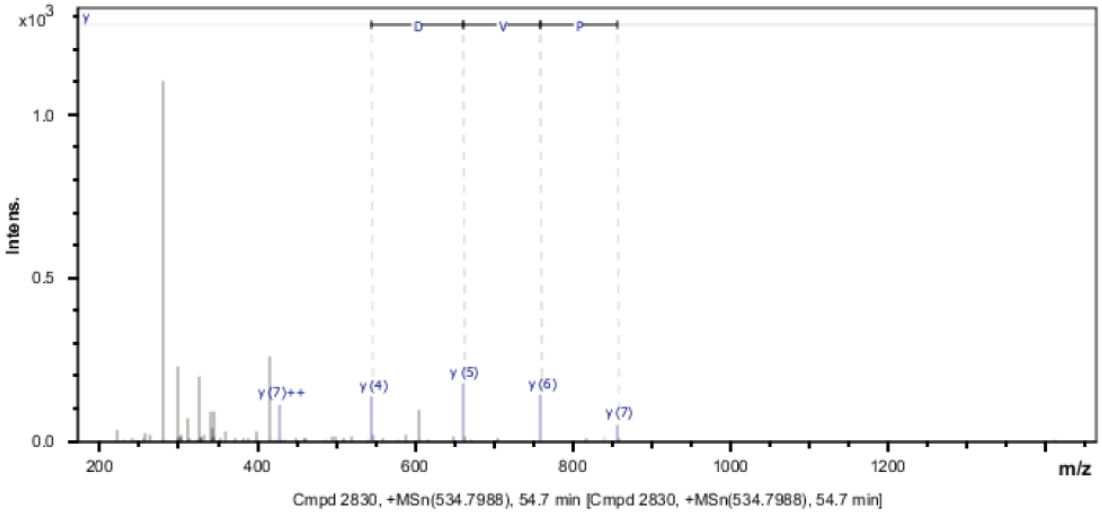
Cmpd 917, +MSn(567.8072), 32.3 min [Cmpd 917, +MSn(567.8072), 32.3 min]

Protein Name: Ras-related protein ARA-3, putative, expressed [Oryza sativa (japonica cultivar-group)]
Accession: gi|108711785 **Parent m/z:** 658.833557, 2+
Sequence: K.LQIWDTAGQER.F
Score: 30.99

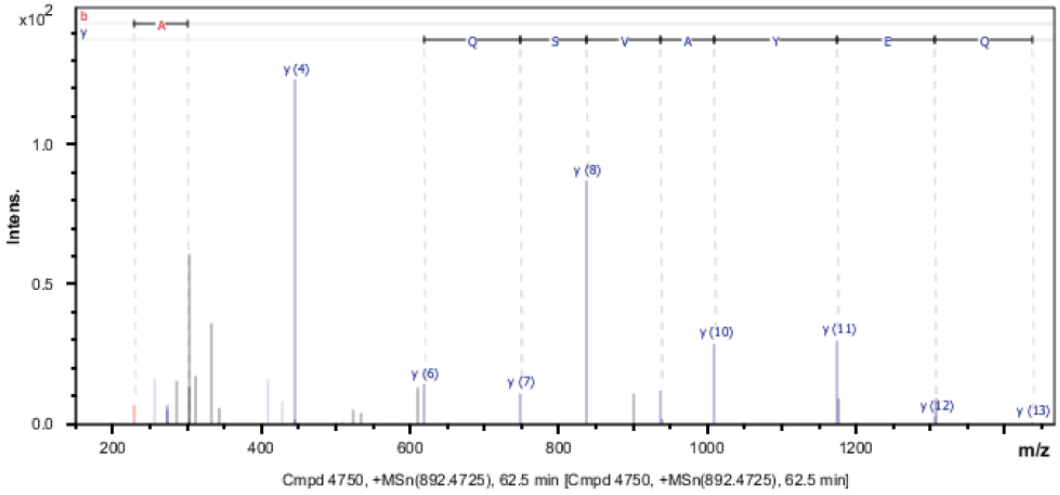


Cmpd 3393, +MSn(658.8373), 57.9 min [Cmpd 3393, +MSn(658.8373), 57.9 min]

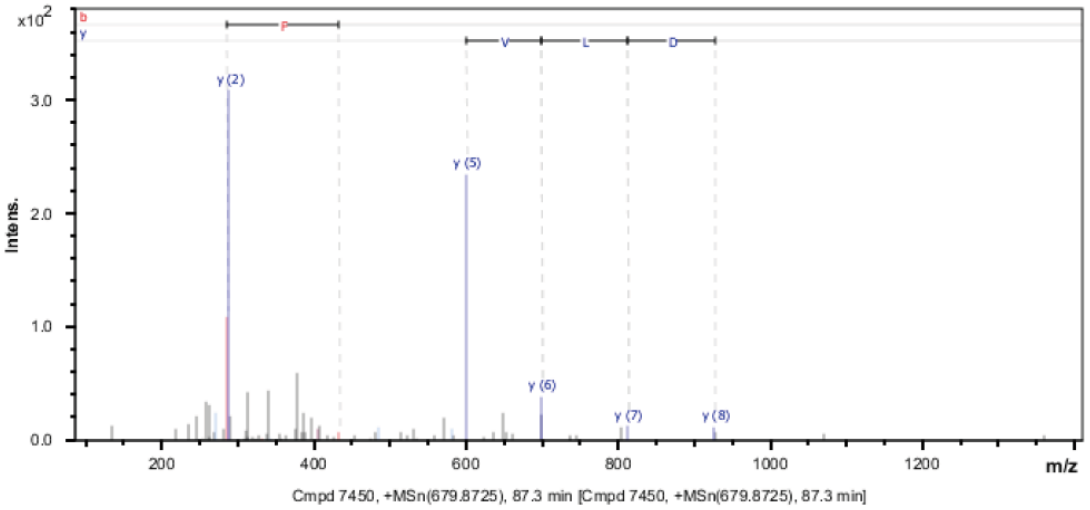
Protein Name: Cyclic nucleotide-gated ion channel 7, putative, expressed [Oryza sativa (japonica cultivar-group)]
Accession: gi|108862663 **Parent m/z:** 534.800232, 2+
Sequence: R.VLPVDLQER.V
Score: 25.55



Protein Name: 1-acyl-sn-glycerol-3-phosphate acyltransferase PLS1, putative, expressed [Oryza sativa (japonica cultivar-group)]
Accession: gi|108864611 **Parent m/z:** 892.484863, 2+
Sequence: K.LLAAQEYAVSQGLPAPR.N
Score: 57.6

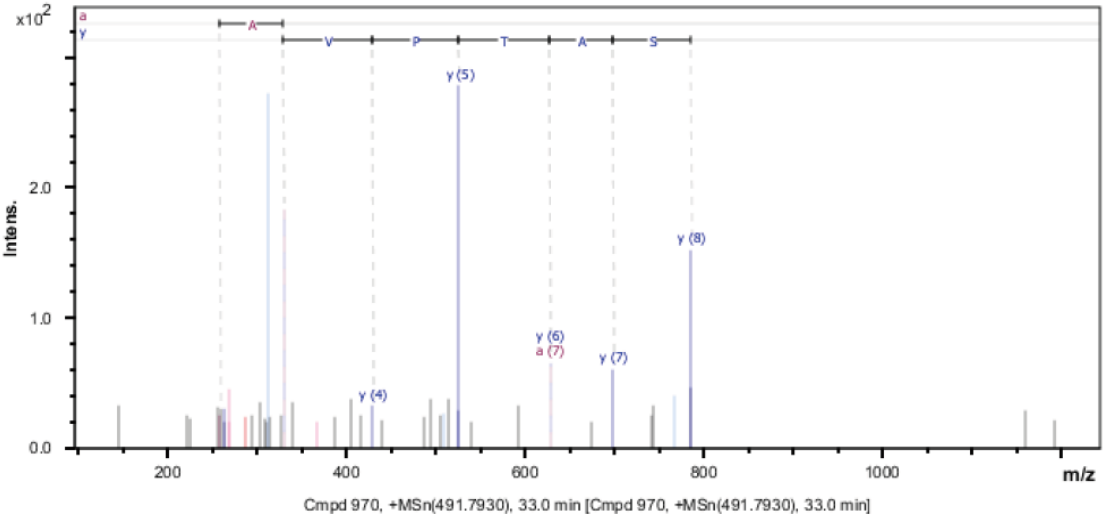


Protein Name: putative glutathione S-transferase OsGSTF4 [Oryza sativa Japonica Group]
Accession: gi|11177843 **Parent m/z:** 679.866577, 2+
Sequence: K.GLDFDLVPVDLR.T
Score: 34.78



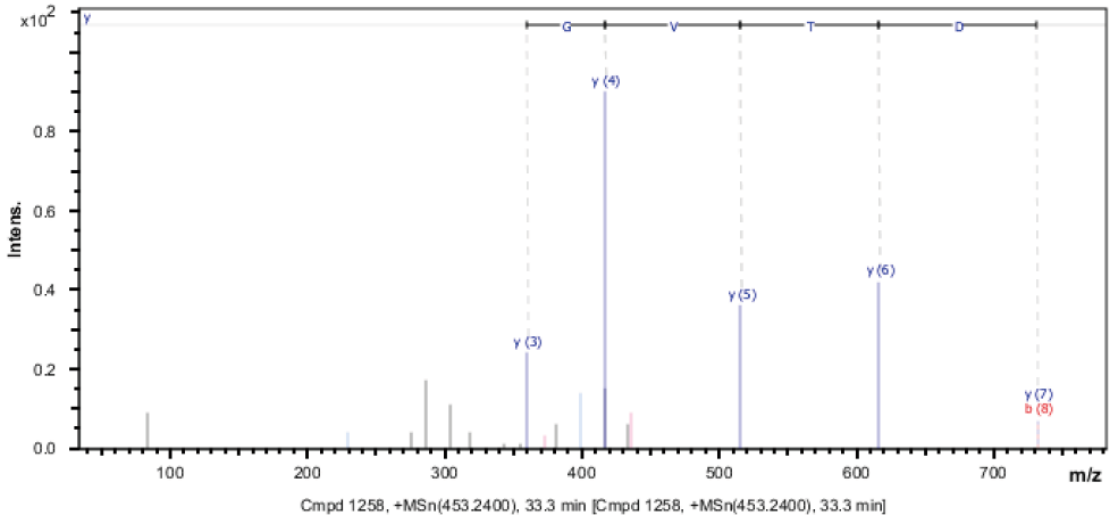
Cmpd 7450, +MSn(679.8725), 87.3 min [Cmpd 7450, +MSn(679.8725), 87.3 min]

Protein Name: Os01g0238600 [Oryza sativa Japonica Group]
Accession: gi|115435560 **Parent m/z:** 491.79304, 2+
Sequence: K.VVSATPVPGR.A
Score: 40.34



Cmpd 970, +MSn(491.7930), 33.0 min [Cmpd 970, +MSn(491.7930), 33.0 min]

Protein Name: Os01g0311700 [Oryza sativa Japonica Group]
Accession: gi|115436212 **Parent m/z:** 453.238037, 2+
Sequence: K.SSDTVGLAR.V
Score: 47.87



Protein Name: Os01g0514300 [Oryza sativa Japonica Group]
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Sequence: R.AVDATDLPATSVR.D
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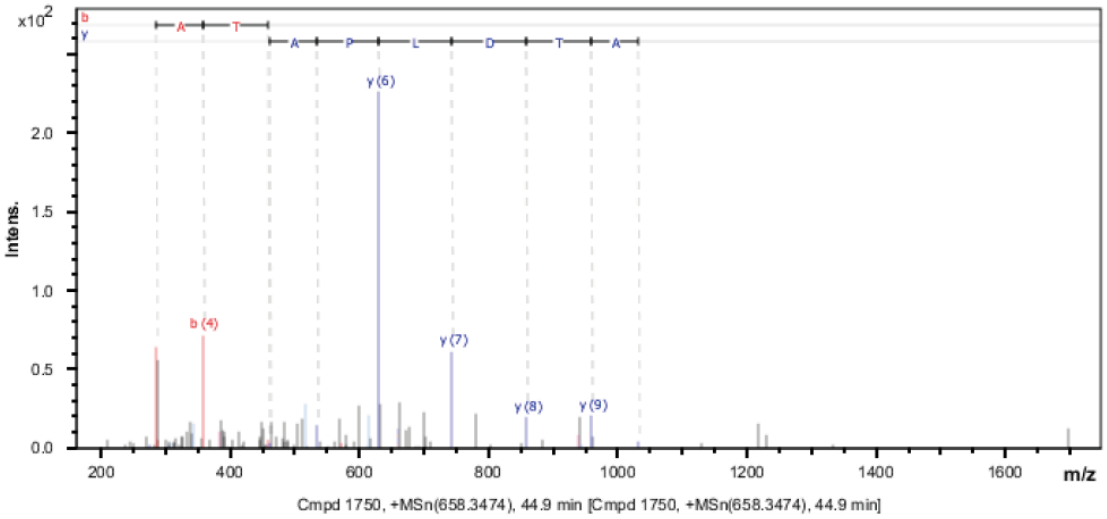
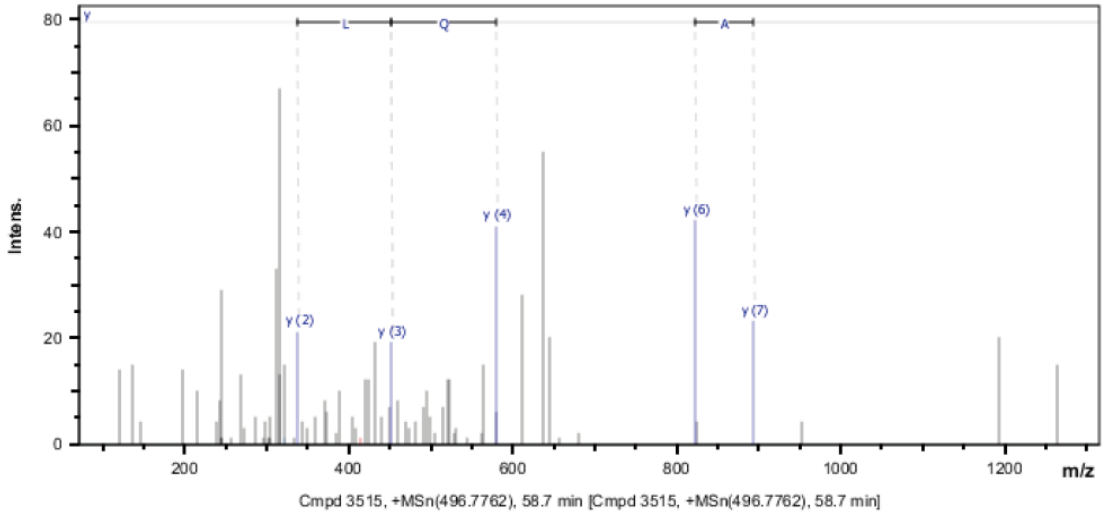


Protein Name: Os01g0575500 [Oryza sativa Japonica Group]
Accession: gi|115437824 **Parent m/z:** 496.765686, 2+
Sequence: K.VAGWQLYR.T
Score: 20.92



Protein Name: Os01g0706700 [Oryza sativa Japonica Group]
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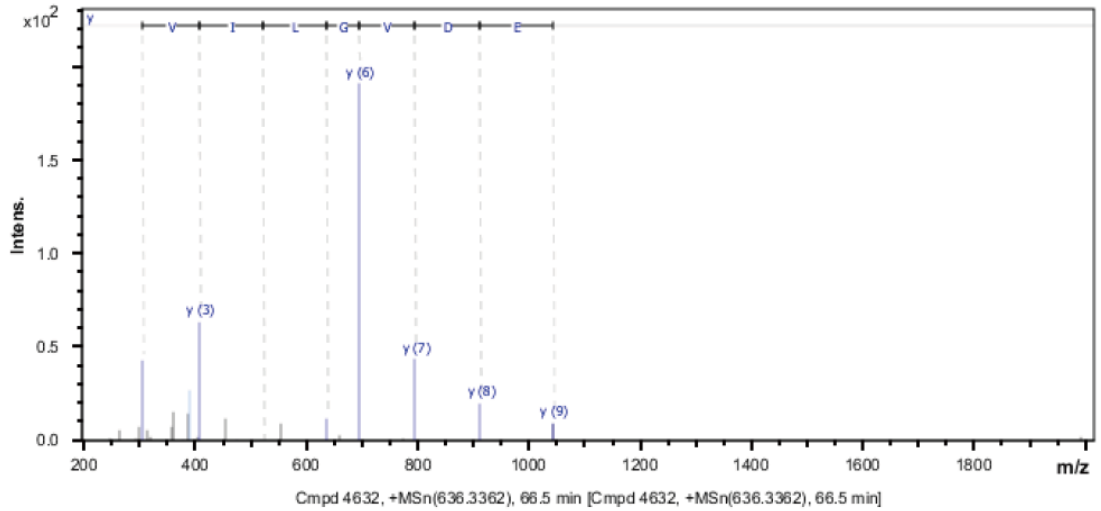
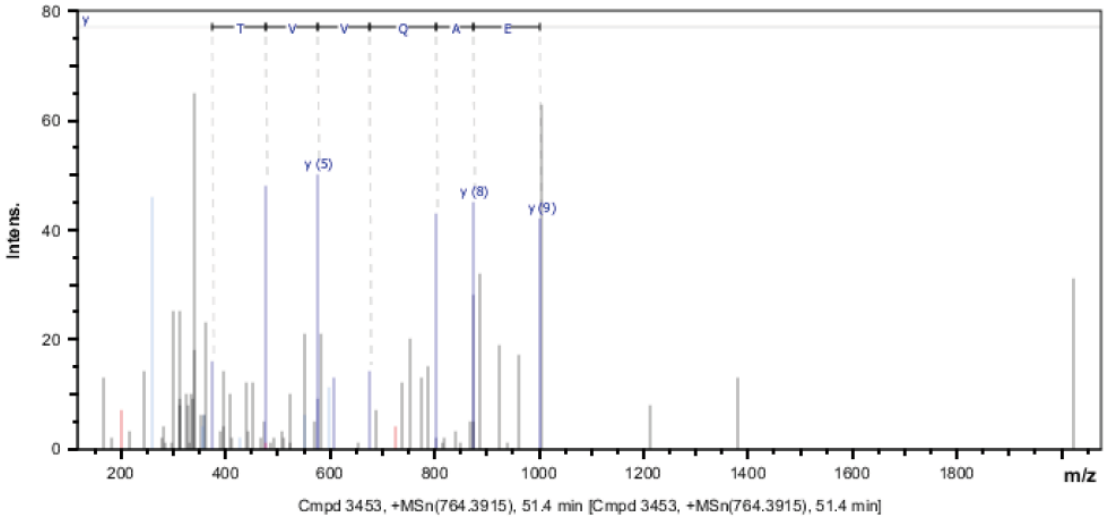
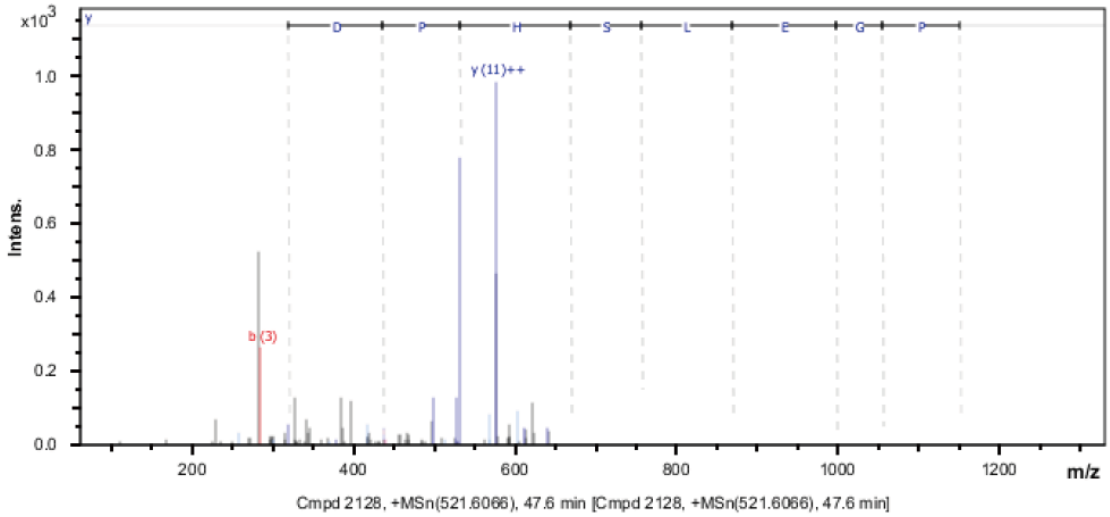
Sequence: R.ELEDVGLIVER.V
Score: 68.32



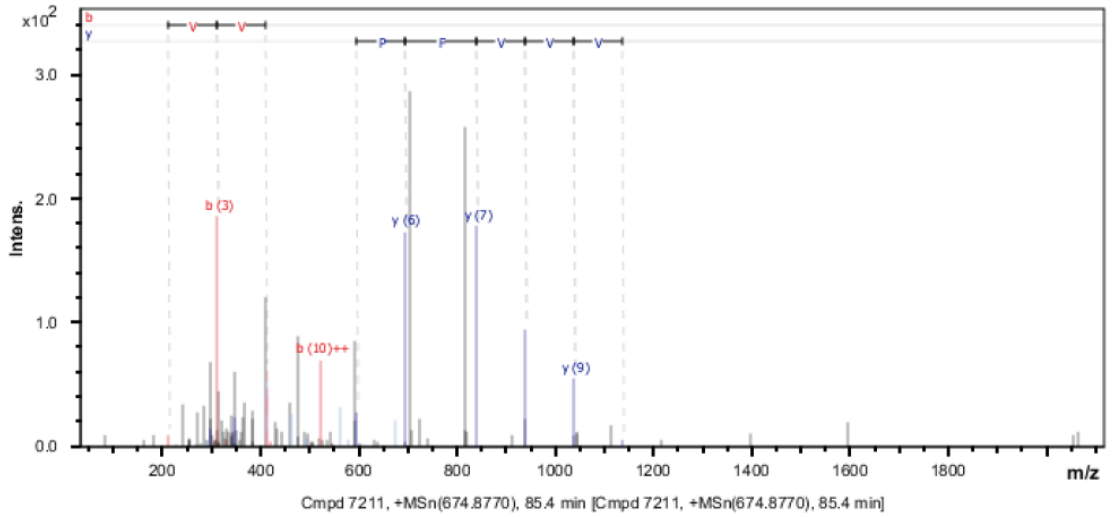
Protein Name: Os01g0706900 [Oryza sativa Japonica Group]
Accession: gi|115439481 **Parent m/z:** 764.398193, 2+
Sequence: R.EADPLEAQVVTVTR.F
Score: 34.46



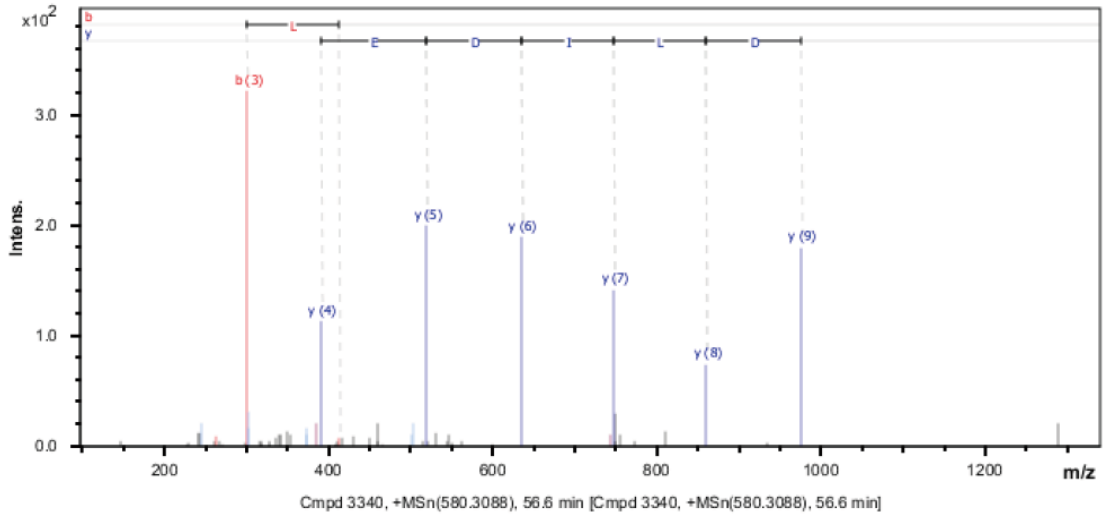
Protein Name: Os01g0975900 [Oryza sativa Japonica Group]
Accession: gi|115442565 **Parent m/z:** 521.611877, 3+
Sequence: R.IAVGAPGELSHPDTAK.A
Score: 59.54



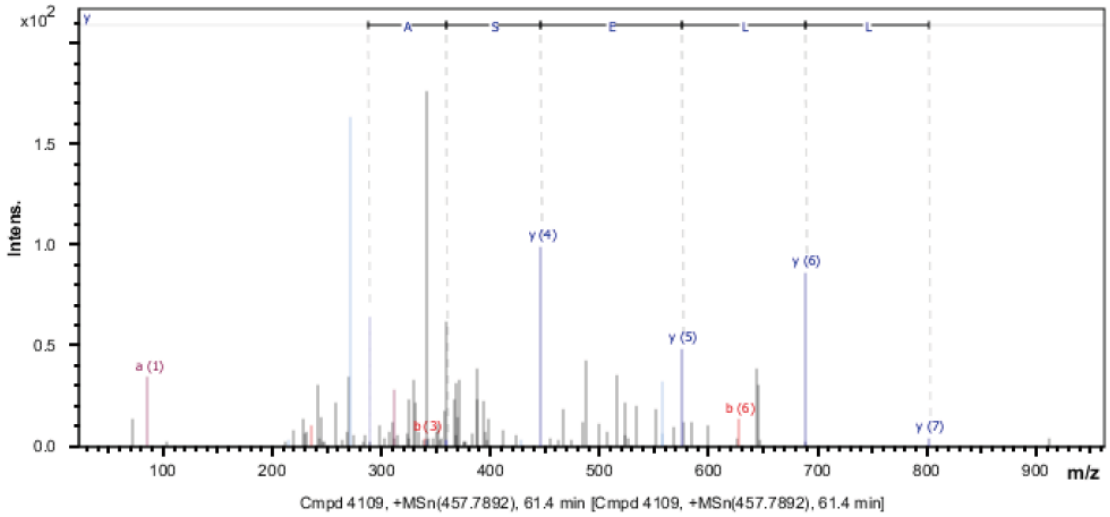
Protein Name: Os01g0978100 [Oryza sativa Japonica Group]
Accession: gi|115442595 **Parent m/z:** 674.879944, 2+
Sequence: K.LVVVVFPSFGER.Y
Score: 25.35



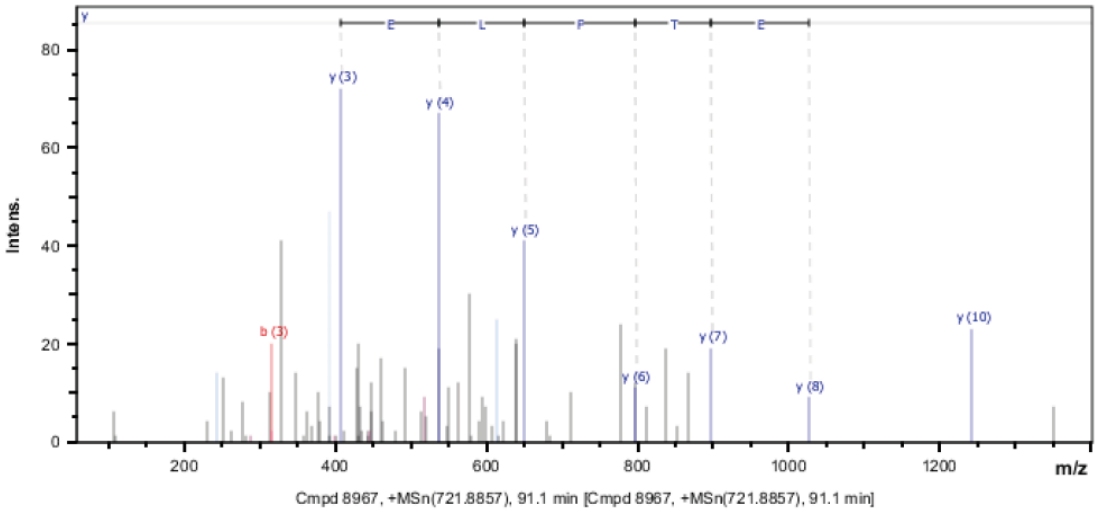
Protein Name: Os02g0526400 [Oryza sativa Japonica Group]
Accession: gi|115446435 **Parent m/z:** 580.303467, 2+
Sequence: K.AIDLIDEAGSR.A
Score: 58.54



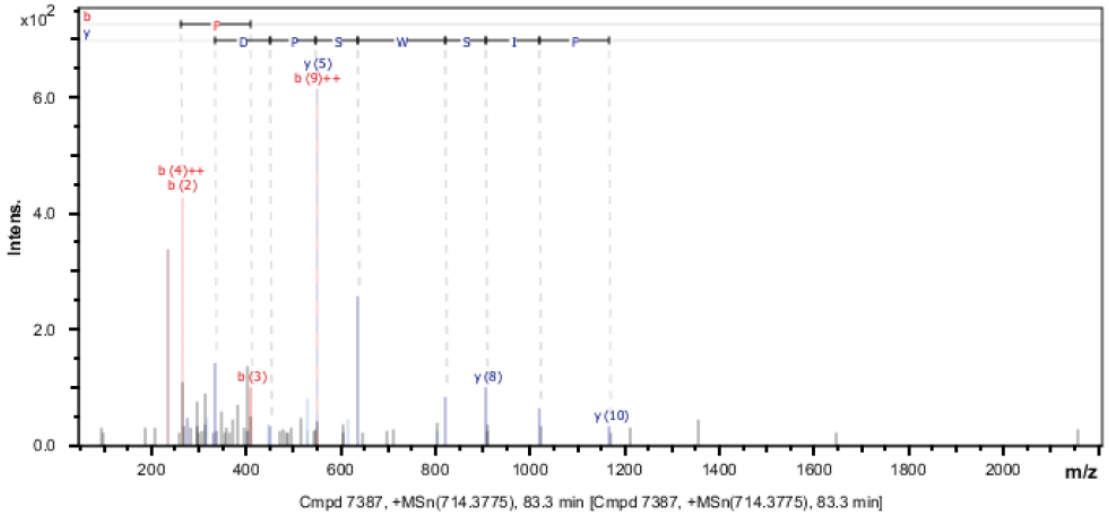
Protein Name: Os03g0136900 [Oryza sativa Japonica Group]
Accession: gi|115450595 **Parent m/z:** 457.782654, 2+
Sequence: R.ILLESAIR.N
Score: 22.72



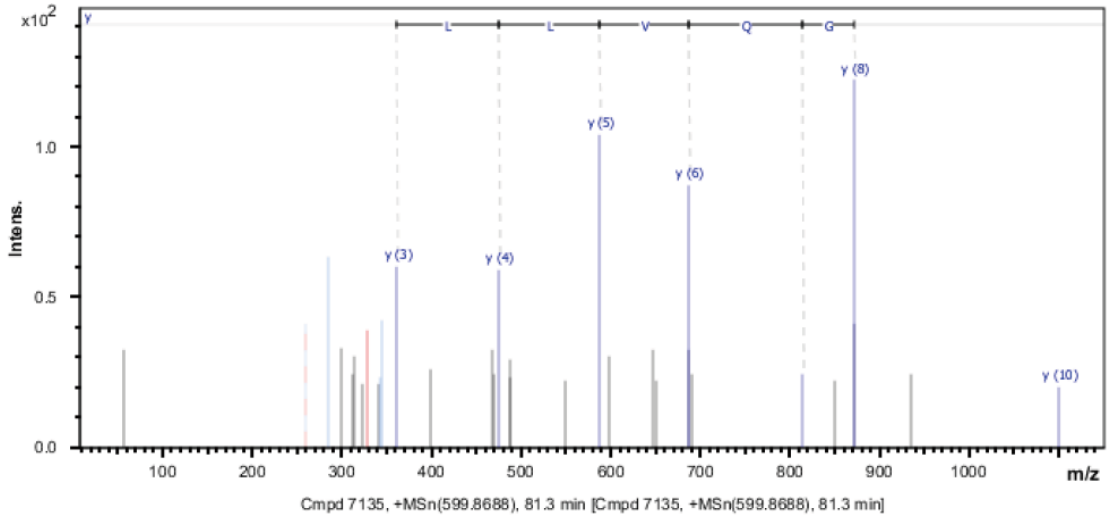
Protein Name: Os03g0819100 [Oryza sativa Japonica Group]
Accession: gi|115456219 **Parent m/z:** 721.875366, 2+
Sequence: K.SIDTETFLEFLK.T
Score: 35.16



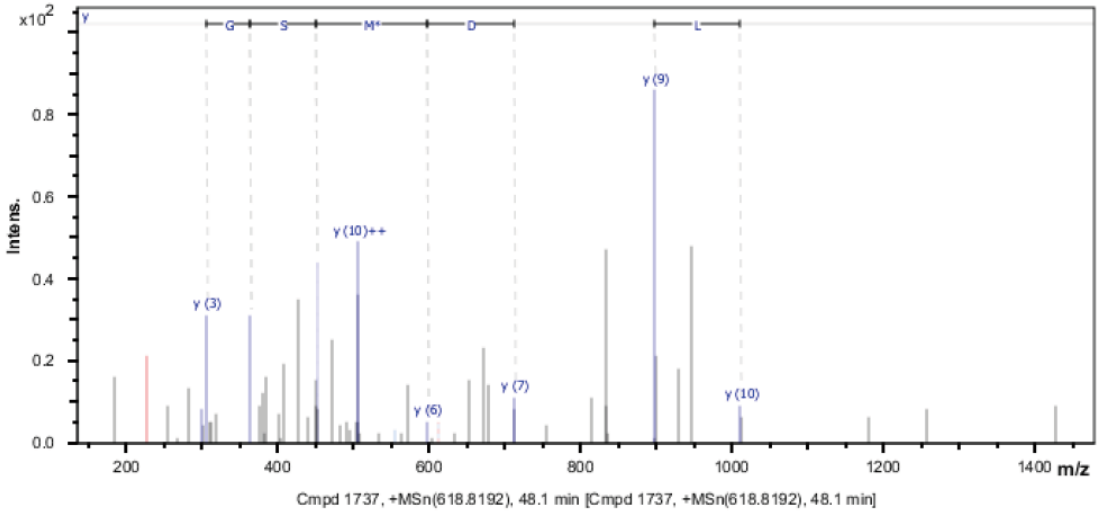
Protein Name: Os04g0555700 [Oryza sativa Japonica Group]
Accession: gi|115459838 **Parent m/z:** 714.37753, 2+
Sequence: K.IFFISWSPDTSK.V
Score: 50.95



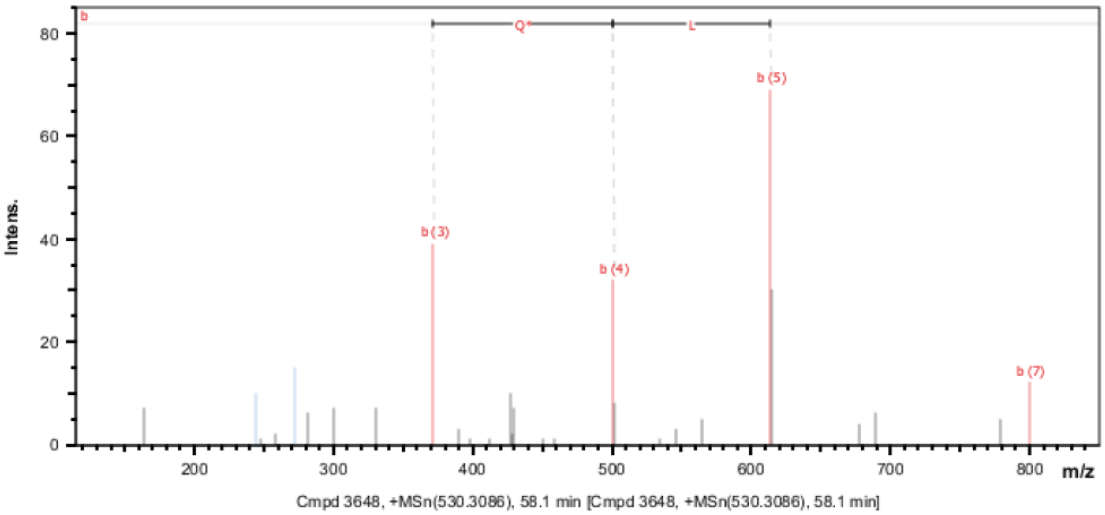
Protein Name: Os05g0169100 [Oryza sativa Japonica Group]
Accession: gi|115462319 **Parent m/z:** 599.86885, 2+
Sequence: R.VDIGQVLLSVR.C
Score: 69.19



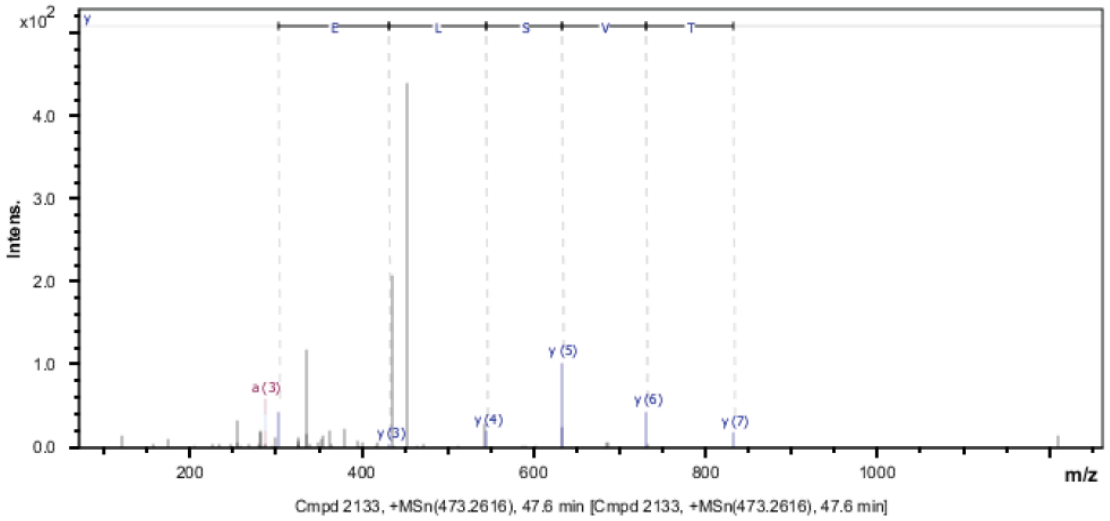
Protein Name: Os05g0560100 [Oryza sativa Japonica Group]
Accession: gi|115465411 **Parent m/z:** 618.807495, 2+
Sequence: K.LLLGEDMSGTGK.G **Modification:** Oxidation: 7
Score: 38.25



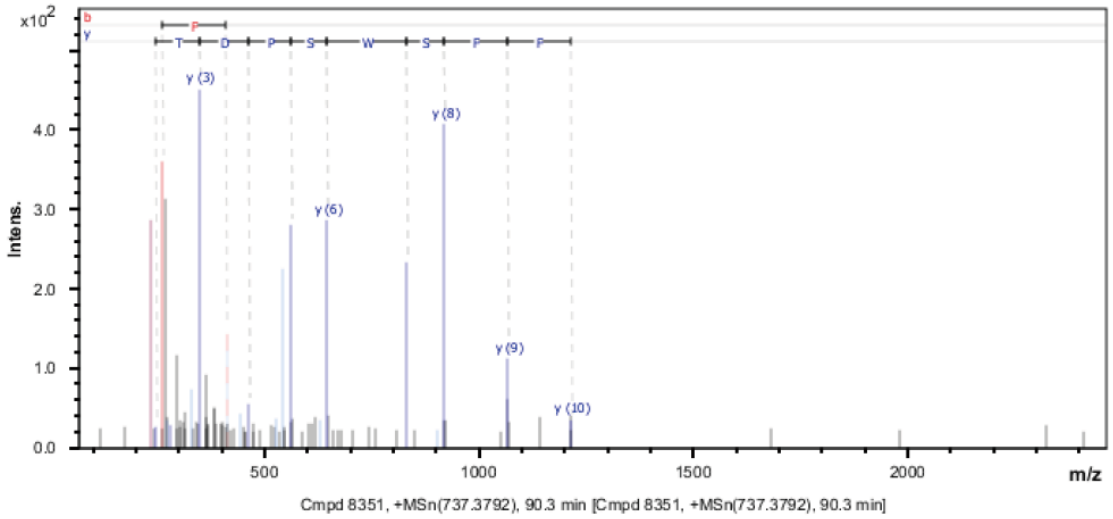
Protein Name: Os07g0109400 [Oryza sativa Japonica Group]
Accession: gi|115470249 **Parent m/z:** 530.304138, 2+
Sequence: K.KLQQLSVNK.D **Modification:** Deamidated: 3, 4
Score: 26.49



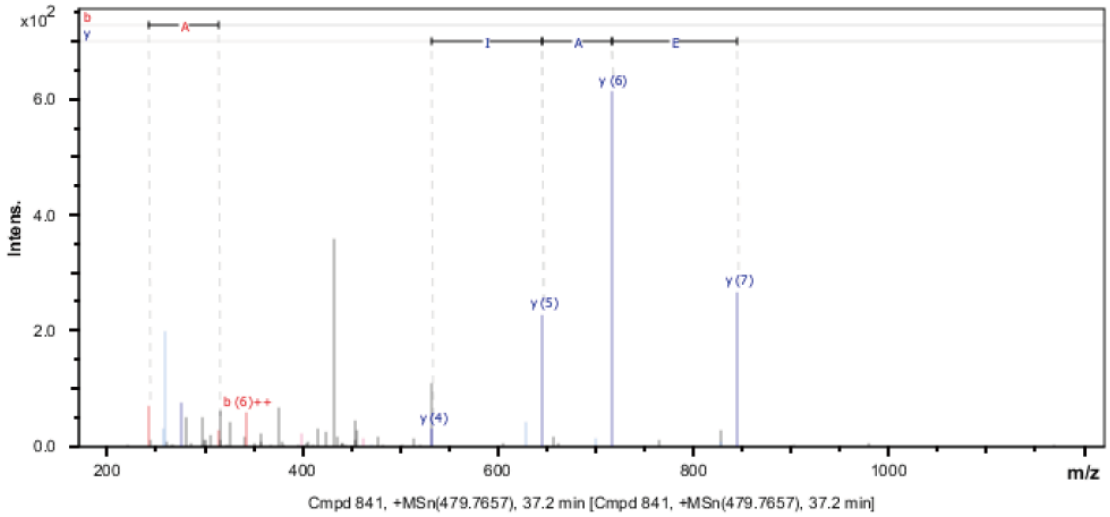
Protein Name: Os07g0205900 [Oryza sativa Japonica Group]
Accession: gi|115471103 **Parent m/z:** 473.266968, 2+
Sequence: K.ITVSLEQR.I
Score: 23.36



Protein Name: Os07g0484200 [Oryza sativa Japonica Group]
Accession: gi|115472099 **Parent m/z:** 737.37922, 2+
Sequence: K.IFFFSWSPDTAR.T
Score: 56.02

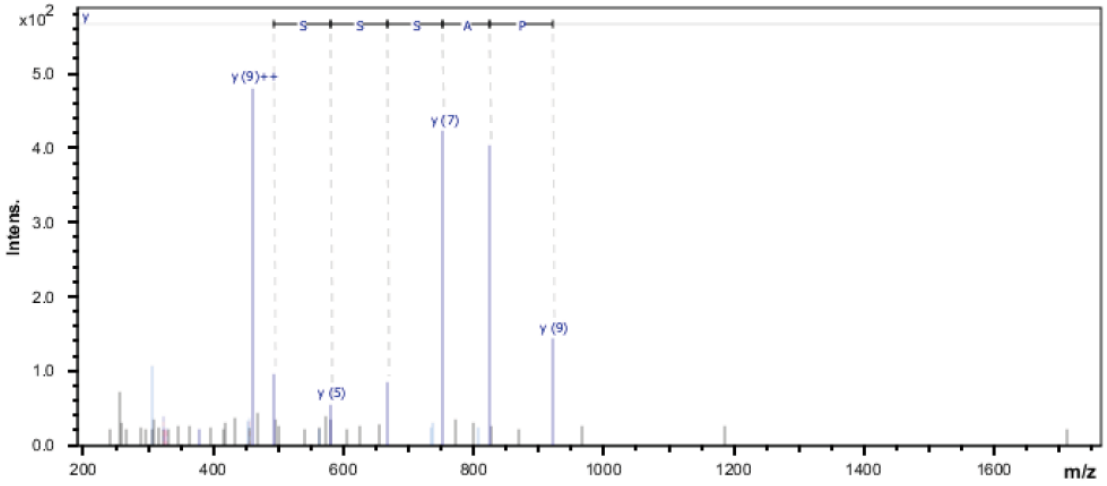


Protein Name: Os07g0570700 [Oryza sativa Japonica Group]
Accession: gi|115472897 **Parent m/z:** 479.769226, 2+
Sequence: K.IEAIQKQK.E **Modification:** Deamidated: 7
Score: 35.89



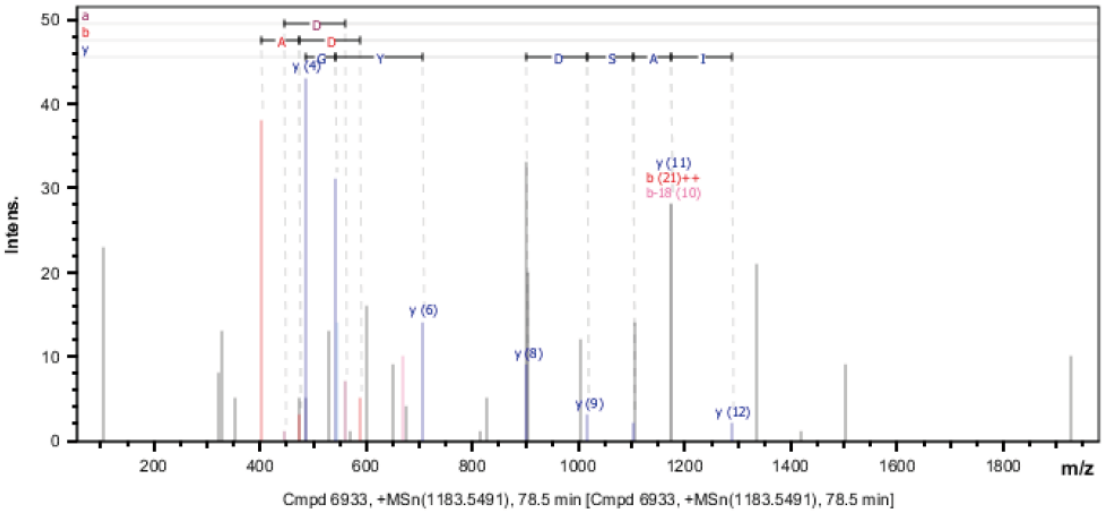
Cmpd 841, +MSn(479.7657), 37.2 min [Cmpd 841, +MSn(479.7657), 37.2 min]

Protein Name: Os07g0680300 [Oryza sativa Japonica Group]
Accession: gi|115474091 **Parent m/z:** 553.31457, 2+
Sequence: M.ALPASSSGLFR.F
Score: 44.98



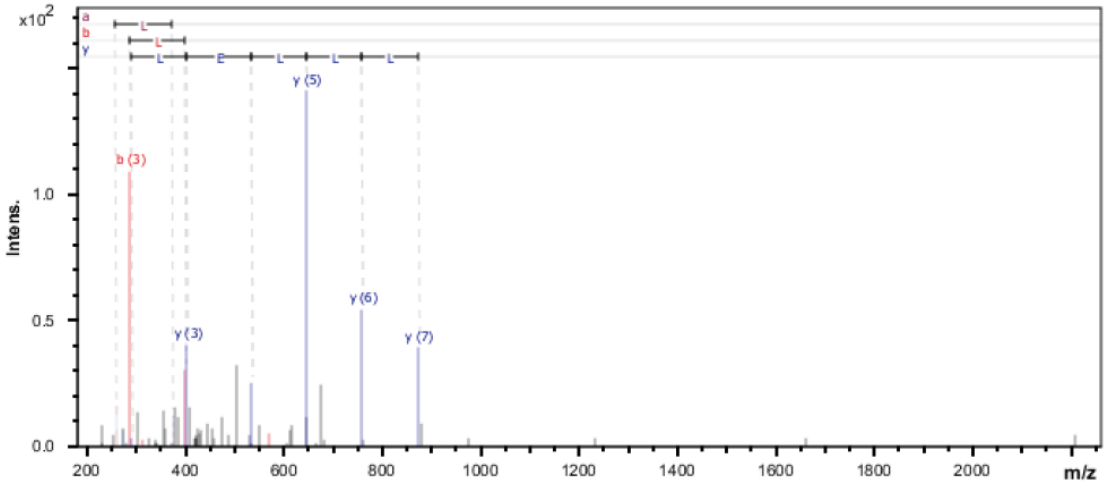
Cmpd 4242, +MSn(553.3146), 58.2 min [Cmpd 4242, +MSn(553.3146), 58.2 min]

Protein Name: Os08g0112800 [Oryza sativa Japonica Group]
Accession: gi|115474475 **Parent m/z:** 1183.565063, 2+
Sequence: K.WTDADVDEFIASDPVYGPQLK.A
Score: 32.63



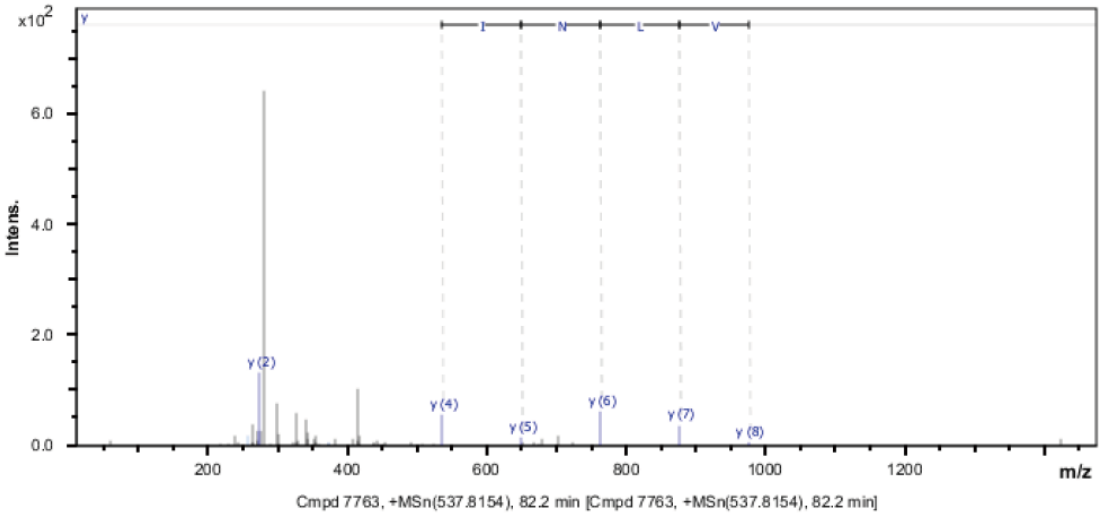
Cmpd 6933, +MSn(1183.5491), 78.5 min [Cmpd 6933, +MSn(1183.5491), 78.5 min]

Protein Name: Os10g0477100 [Oryza sativa Japonica Group]
Accession: gi|115482522 **Parent m/z:** 578.834534, 2+
Sequence: R.DGILLLELDR.L
Score: 40.49

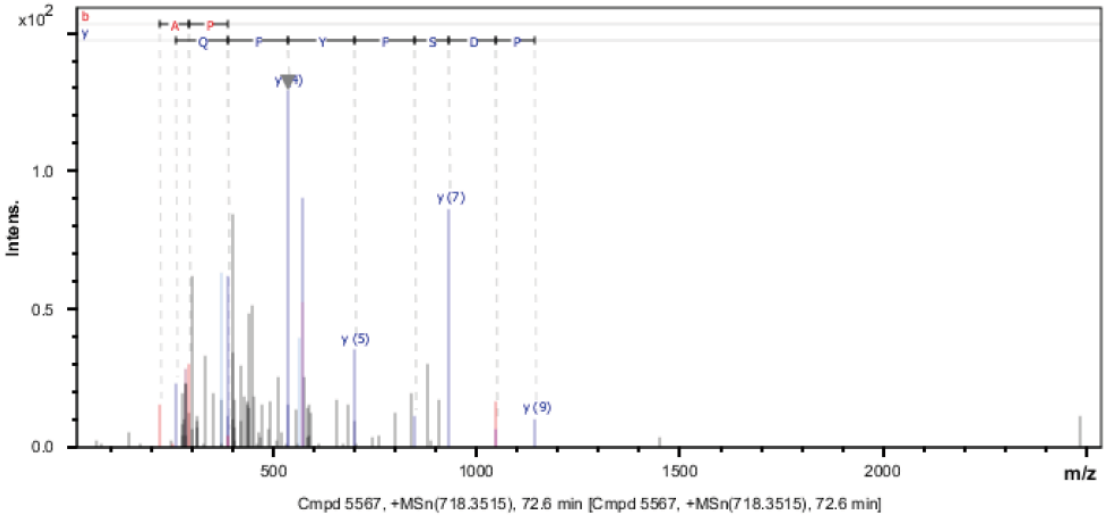


Cmpd 7748, +MSn(578.8411), 82.6 min [Cmpd 7748, +MSn(578.8411), 82.6 min]

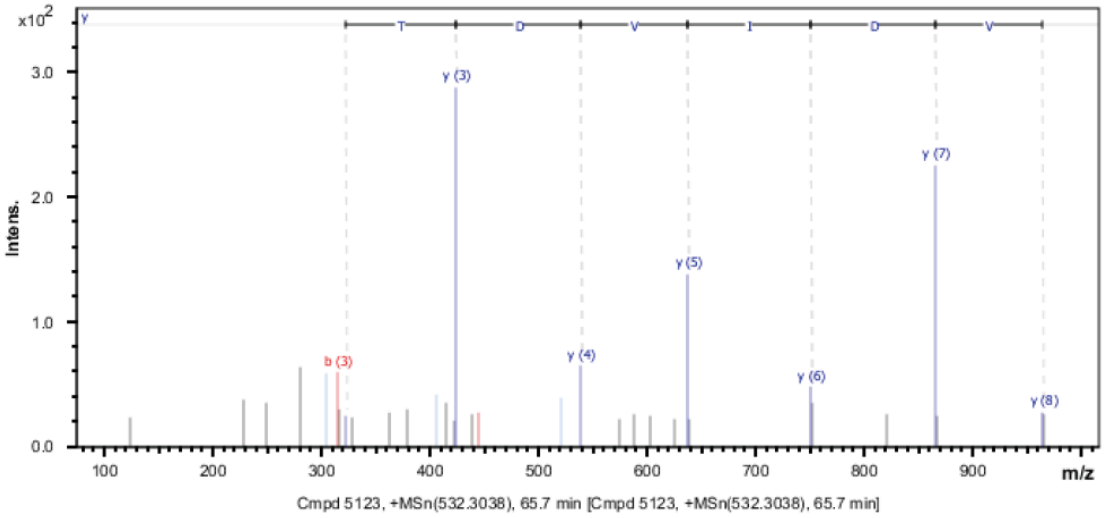
Protein Name: Os10g0569200 [Oryza sativa Japonica Group]
Accession: gi|115483552 **Parent m/z:** 537.808533, 2+
Sequence: R.VVLNIFDVR.T
Score: 32.37



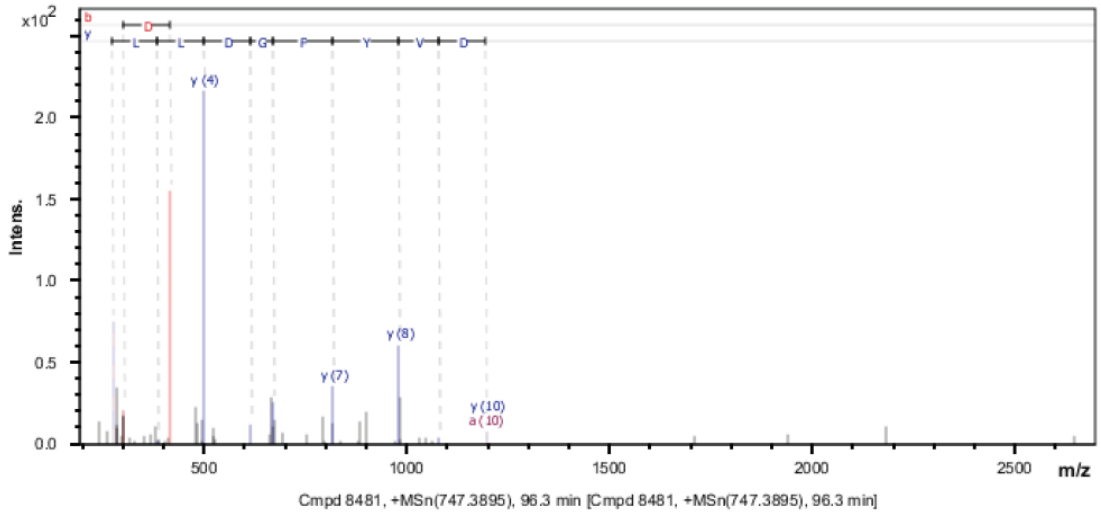
Protein Name: Os11g0153200 [Oryza sativa Japonica Group]
Accession: gi|115484229 **Parent m/z:** 718.348145, 2+
Sequence: K.YGAPDSFYFQLK.R
Score: 45.39



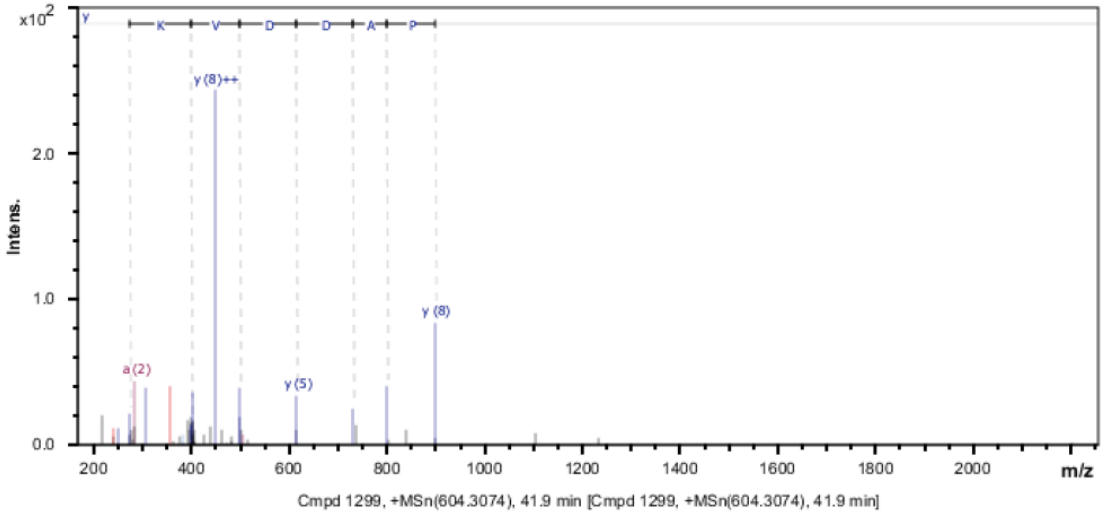
Protein Name: Os11g0660500 [Oryza sativa Japonica Group]
Accession: gi|115486523 **Parent m/z:** 532.30378, 2+
Sequence: K.VVDIVDTFR.L
Score: 53.84



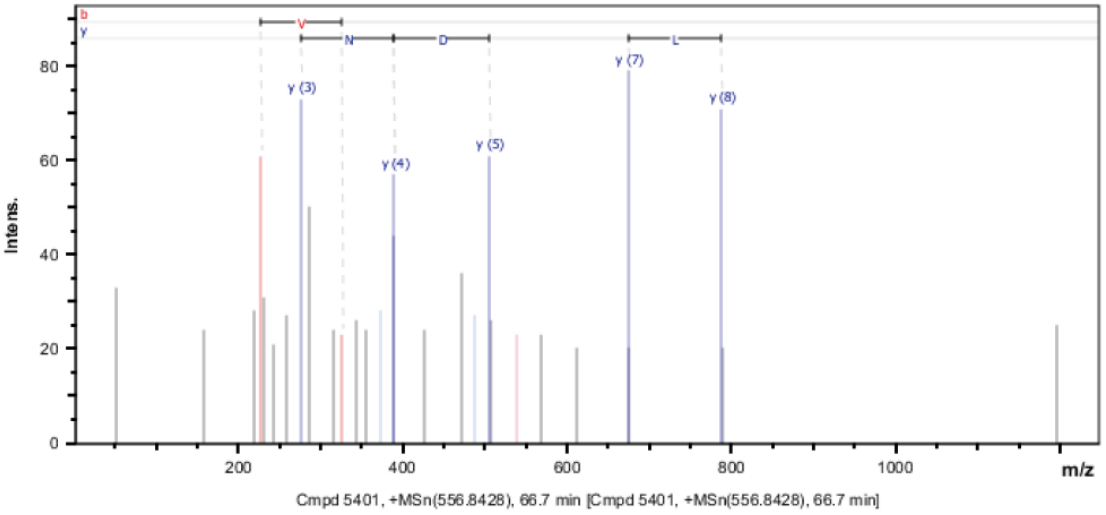
Protein Name: Os12g0578200 [Oryza sativa Japonica Group]
Accession: gi|115489266 **Parent m/z:** 747.394775, 2+
Sequence: R.IWDVYFGDLLPR.L
Score: 52.85



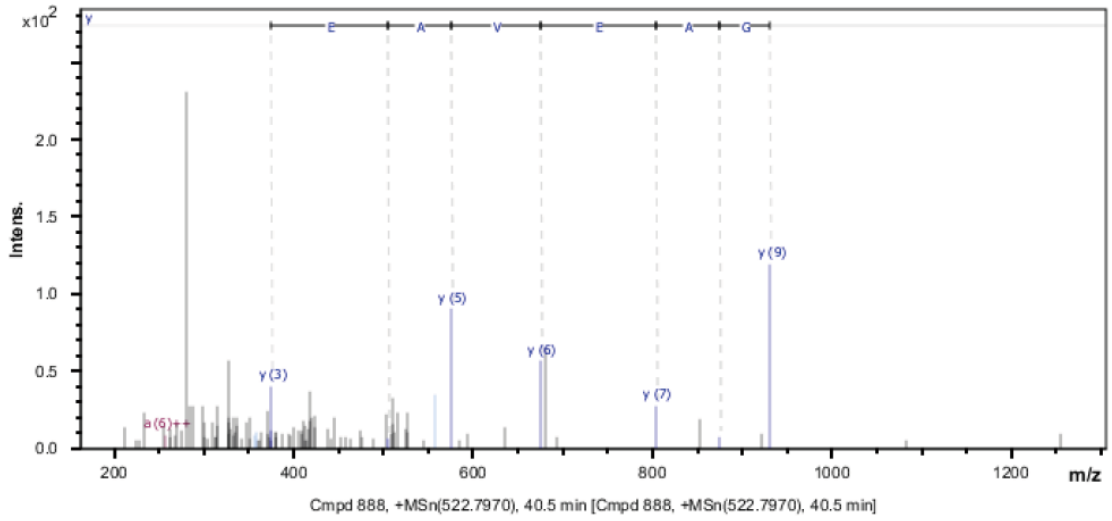
Protein Name: H0305E08.7 [Oryza sativa (indica cultivar-group)]
Accession: gi|116310429 **Parent m/z:** 604.305725, 2+
Sequence: K.FYPADDVKPR.Q
Score: 50.96



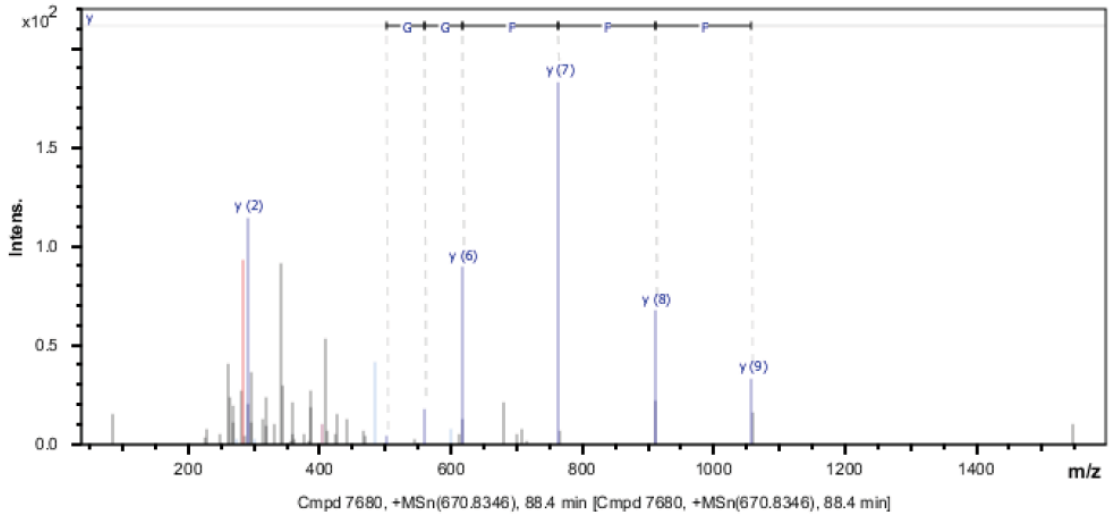
Protein Name: hypothetical protein OsI_28443 [Oryza sativa Indica Group]
Accession: gi|125560755 **Parent m/z:** 556.8428, 2+
Sequence: R.ILVLGLDNAGK.T
Score: 39.13



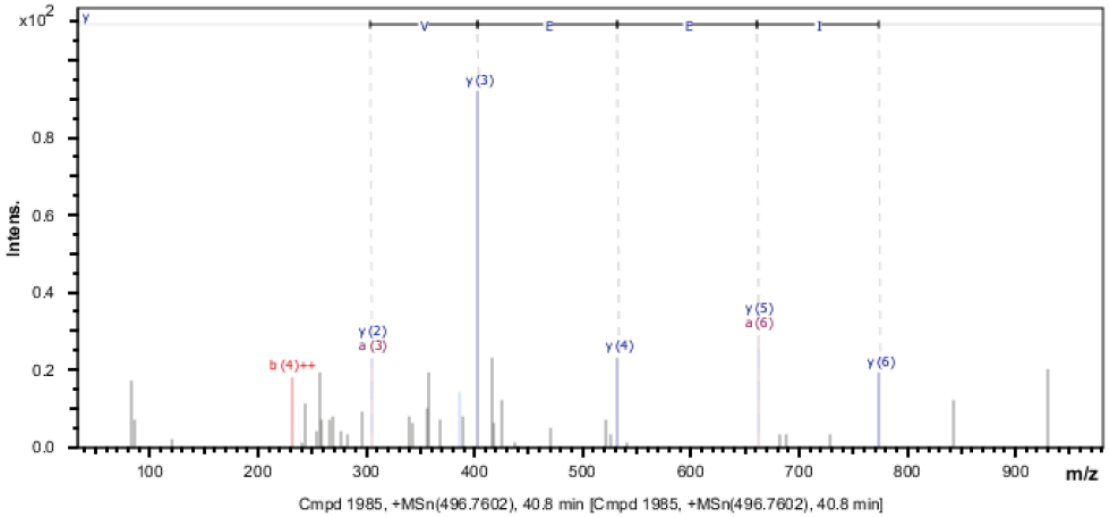
Protein Name: hypothetical protein OsI_29418 [Oryza sativa Indica Group]
Accession: gi|125561725 **Parent m/z:** 522.782776, 2+
Sequence: K.IGAEVAEVTR.V
Score: 35.08



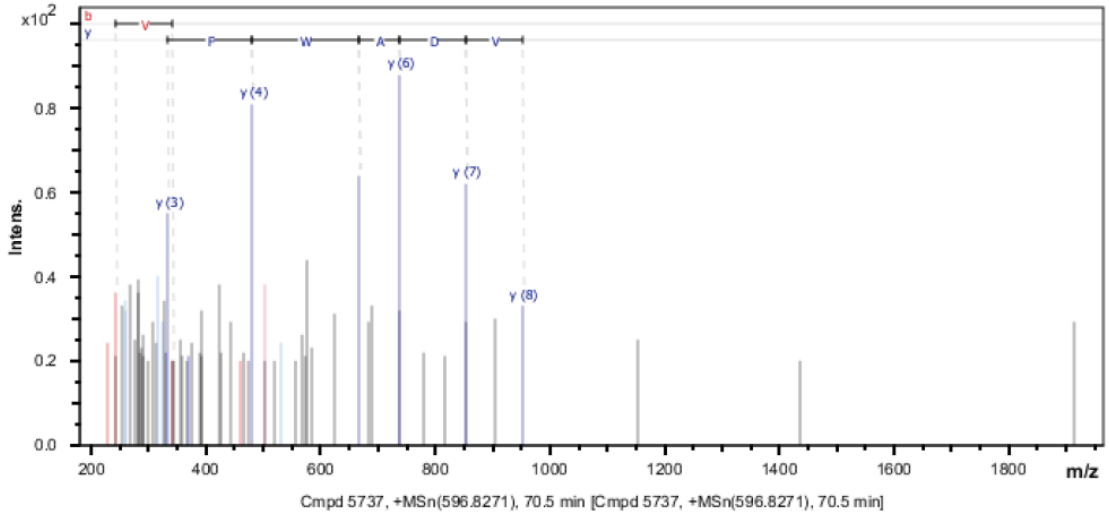
Protein Name: Na+/H+ antiporter-like protein [Oryza sativa Japonica Group]
Accession: gi|15128390 **Parent m/z:** 670.8396, 2+
Sequence: R.VALFFFGGPDDR.E
Score: 49.19



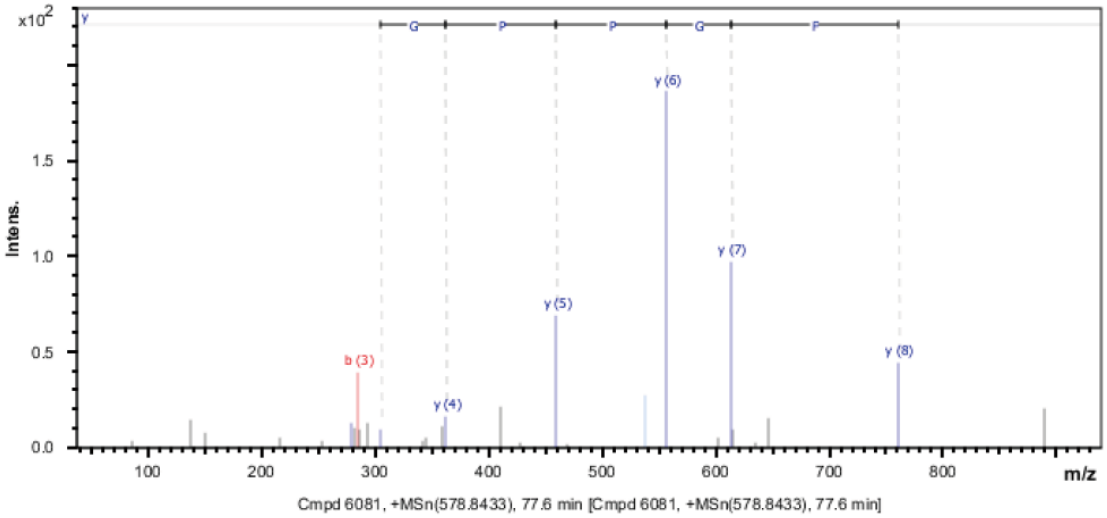
Protein Name: putative 62.8 kDa protein [Oryza sativa Japonica Group]
Accession: gi|19387282 **Parent m/z:** 496.761902, 2+
Sequence: K.AFIEEVER.S
Score: 34.85



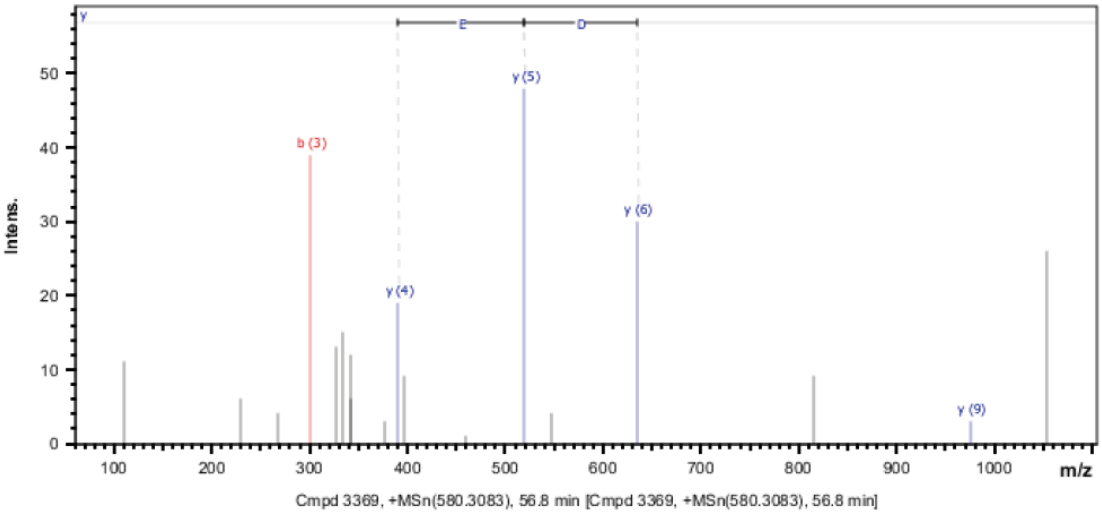
Protein Name: YK426 [Oryza sativa (japonica cultivar-group)]
Accession: gi|2058273 **Parent m/z:** 596.82715, 2+
Sequence: K.LQVDAWFGTR.R
Score: 49.54



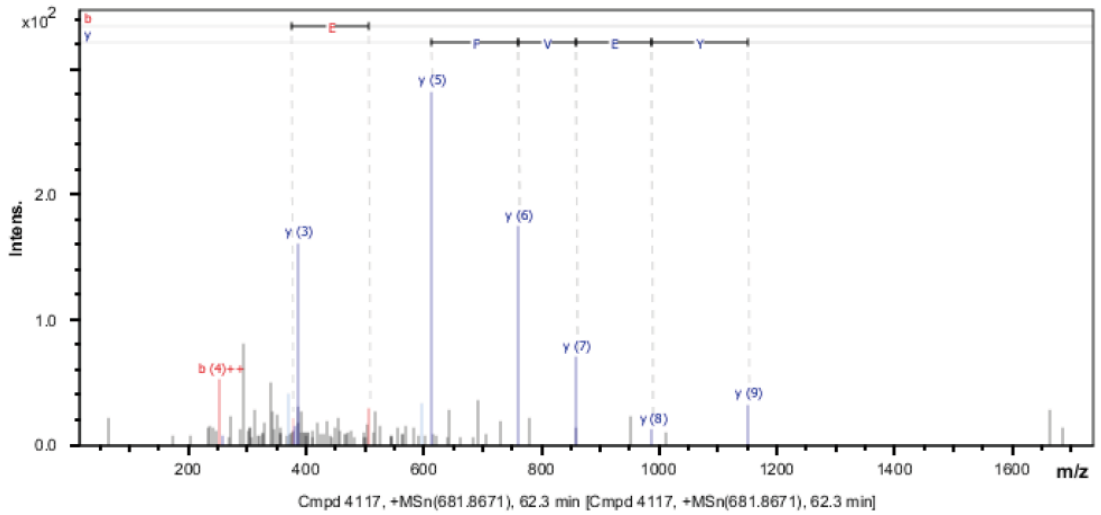
Protein Name: hypothetical protein OsI_36923 [Oryza sativa Indica Group]
Accession: gi|218186155 **Parent m/z:** 578.839905, 2+
Sequence: K.GILLFGPPGTGK.T
Score: 40.45



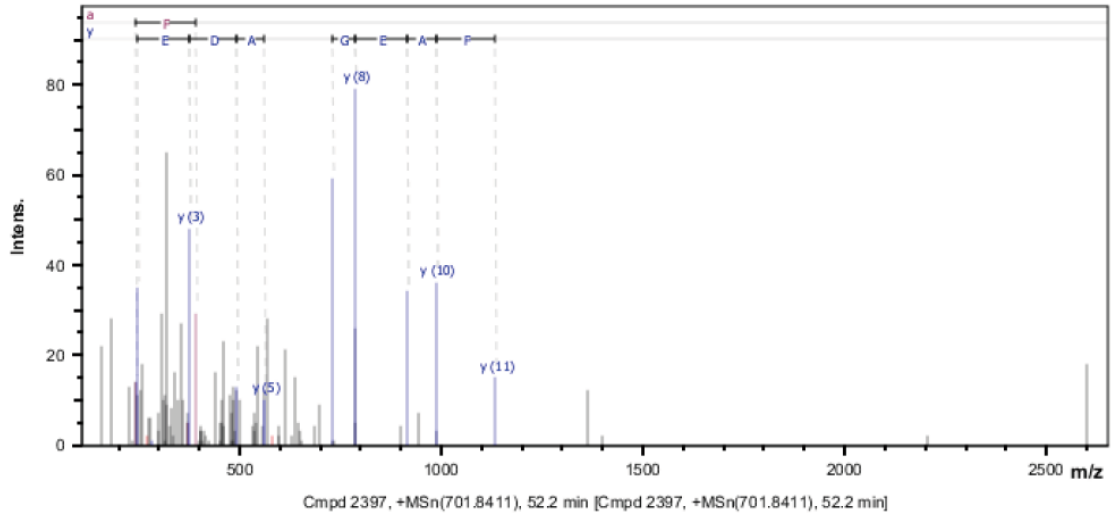
Protein Name: hypothetical protein OsI_37876 [Oryza sativa Indica Group]
Accession: gi|218186618 **Parent m/z:** 580.304993, 2+
Sequence: K.AIDLIDEAGSR.V
Score: 24.3



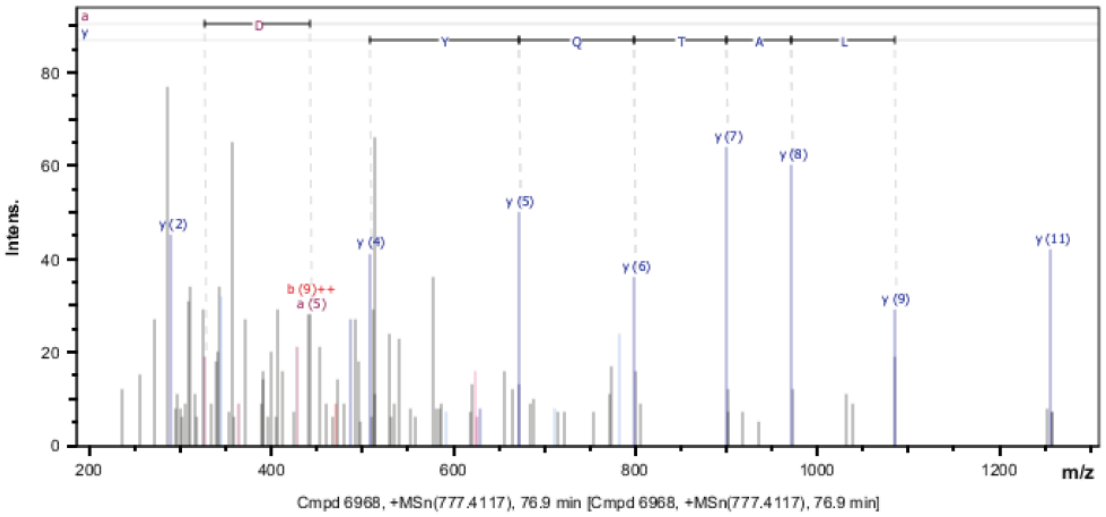
Protein Name: hypothetical protein OsI_10808 [Oryza sativa Indica Group]
Accession: gi|218192466 **Parent m/z:** 681.851379, 2+
Sequence: R.VIYEVFPEPNR.Q
Score: 46.05



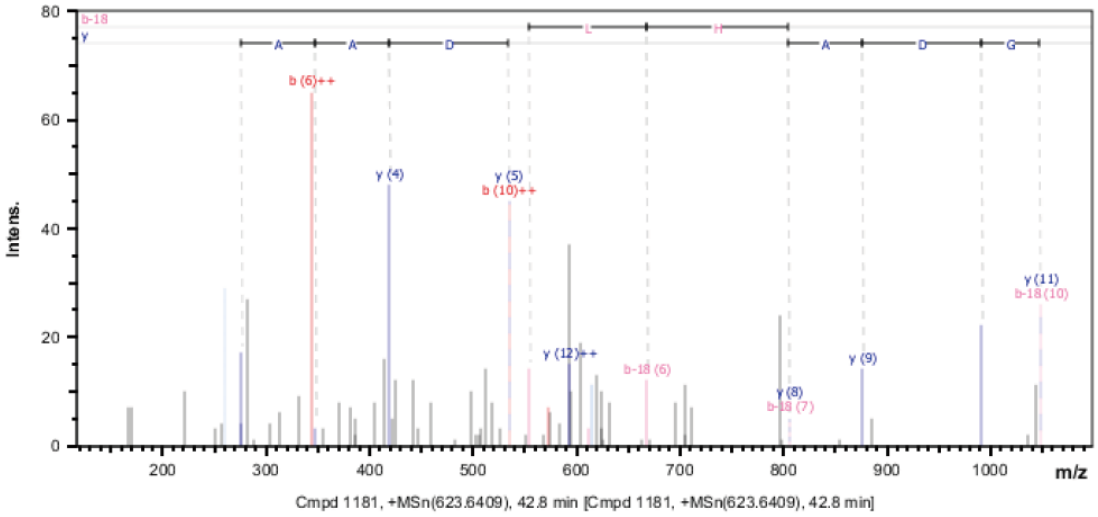
Protein Name: hypothetical protein OsI_23301 [Oryza sativa Indica Group]
Accession: gi|218198351 **Parent m/z:** 701.846191, 2+
Sequence: R.VAVFAEGPAADEAR.A
Score: 50.44



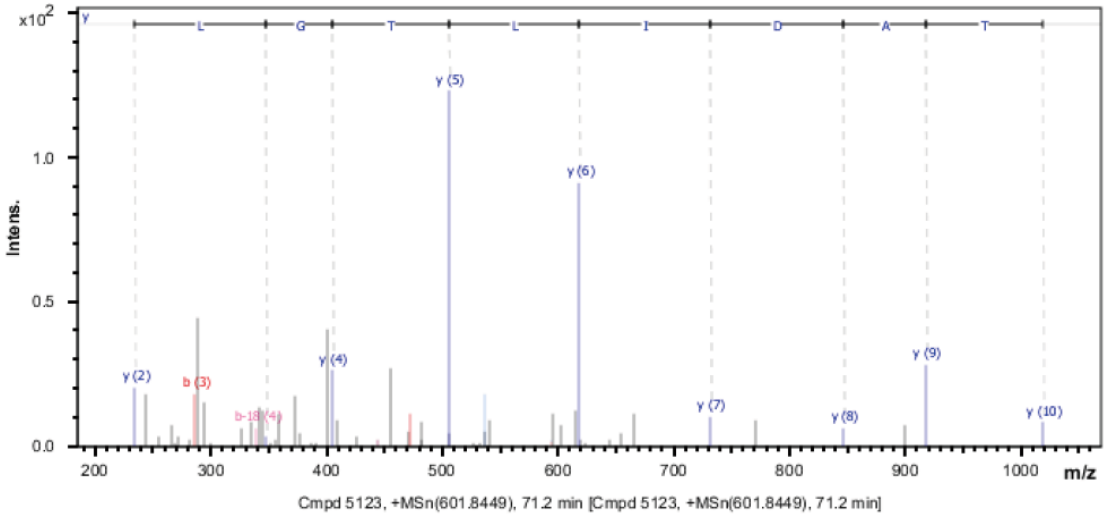
Protein Name: hypothetical protein OsI_24243 [Oryza sativa Indica Group]
Accession: gi|218198784 **Parent m/z:** 777.400757, 2+
Sequence: R.LAIGDLATQYFADR.D
Score: 48.21



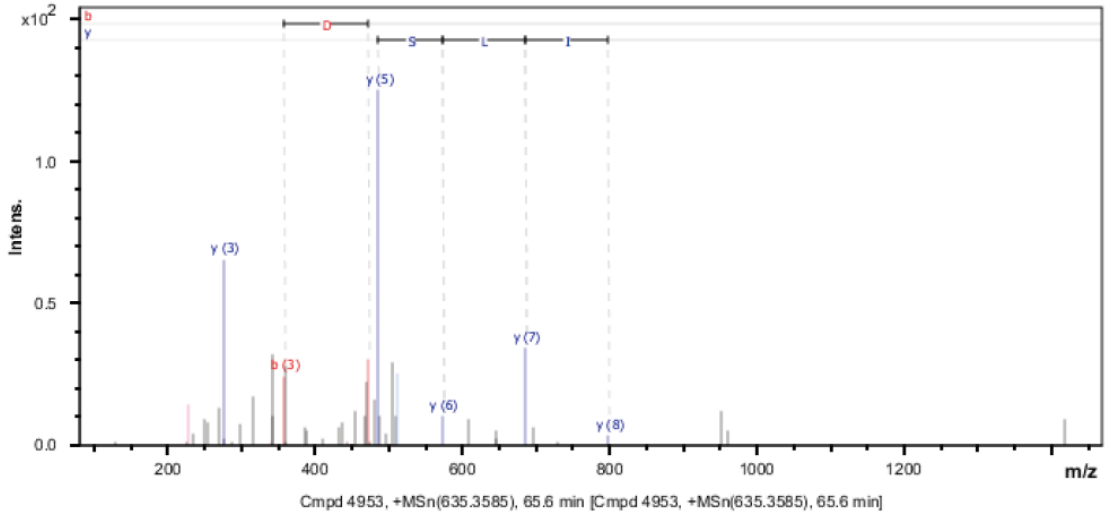
Protein Name: hypothetical protein OsJ_11136 [Oryza sativa Japonica Group]
Accession: gi|222625063 **Parent m/z:** 623.643555, 3+
Sequence: R.IQDNTLHGDAATVDAATR.D
Score: 28.2



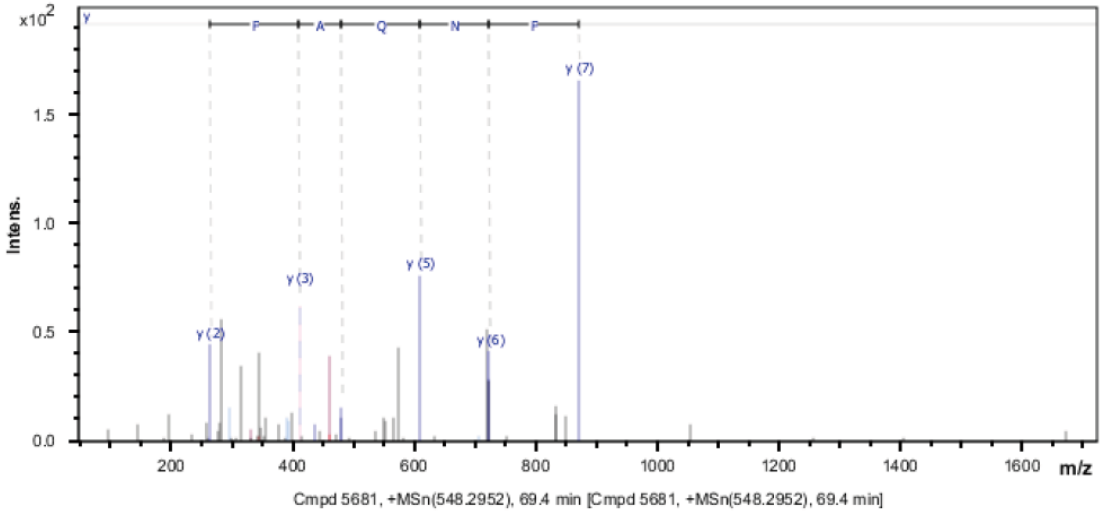
Protein Name: Unknown protein [Oryza sativa Japonica Group]
Accession: gi|24476037 **Parent m/z:** 601.849915, 2+
Sequence: K.LATADILTGLSK.N
Score: 54.23



Protein Name: Putative GDP-mannose pyrophosphorylase [Oryza sativa Japonica Group]
Accession: gi|26006499 **Parent m/z:** 635.353455, 2+
Sequence: R.LDQDILSPLAGK.K
Score: 34.33

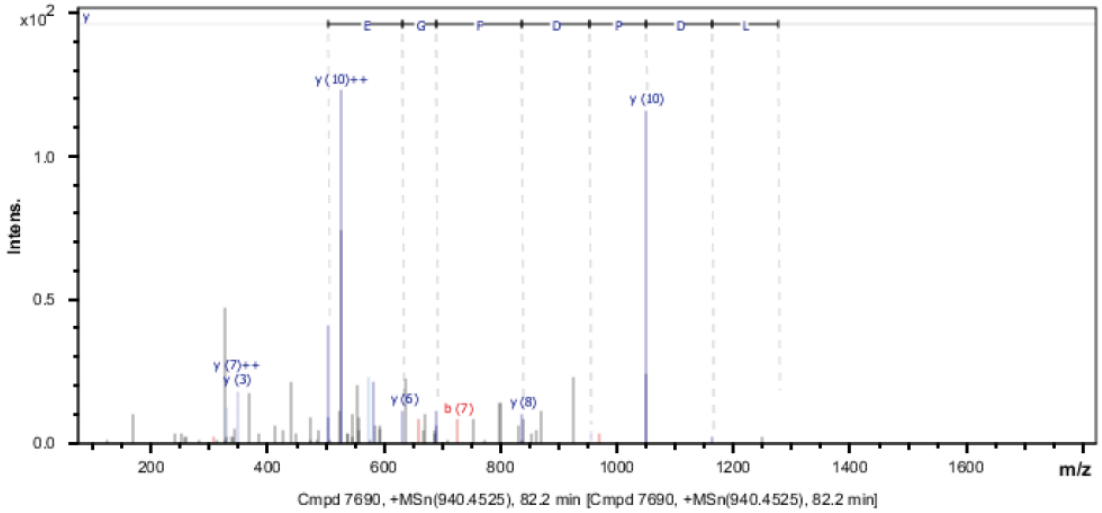


Protein Name: Putative callose synthase [Oryza sativa Japonica Group]
Accession: gi|27452908 **Parent m/z:** 548.30011, 2+
Sequence: R.LLFNQAFSR.G
Score: 25.27



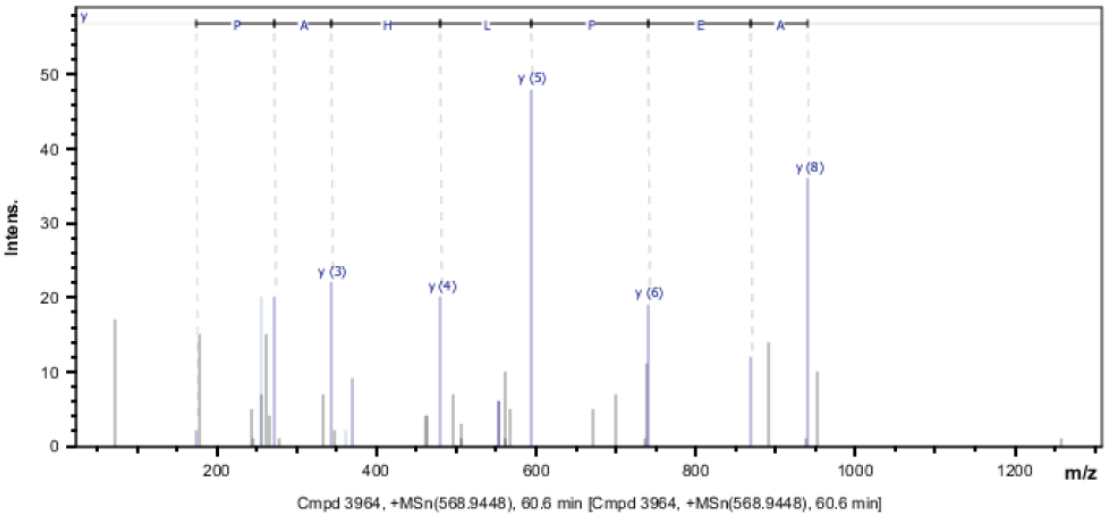
Cmpd 5681, +MSn(548.2952), 69.4 min [Cmpd 5681, +MSn(548.2952), 69.4 min]

Protein Name: invertase [Oryza sativa Indica Group]
Accession: gi|27948558 **Parent m/z:** 940.440613, 3+
Sequence: R.AVPLDDNNEAFEEVLDPDFGESAIGR.V
Score: 30.56

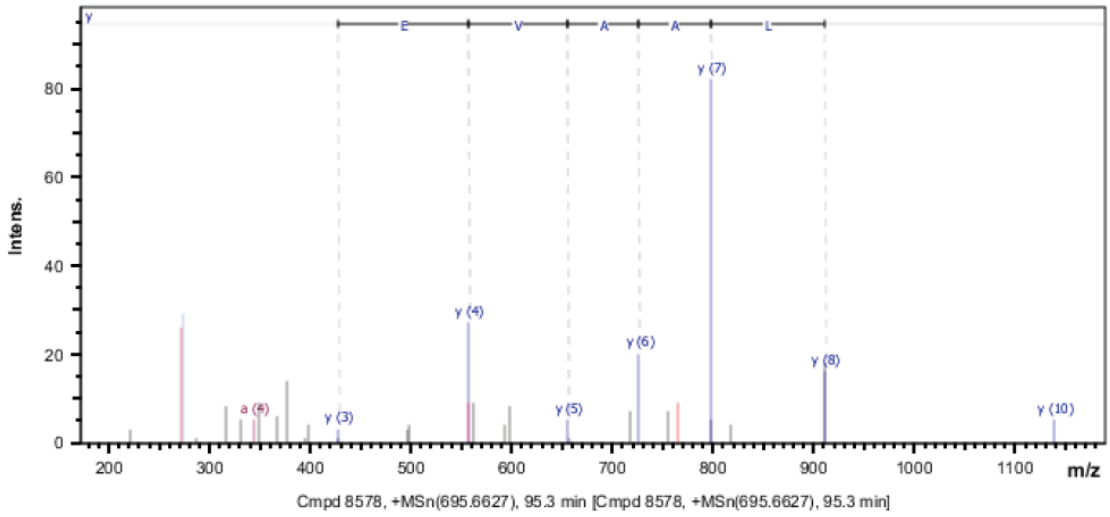


Cmpd 7690, +MSn(940.4525), 82.2 min [Cmpd 7690, +MSn(940.4525), 82.2 min]

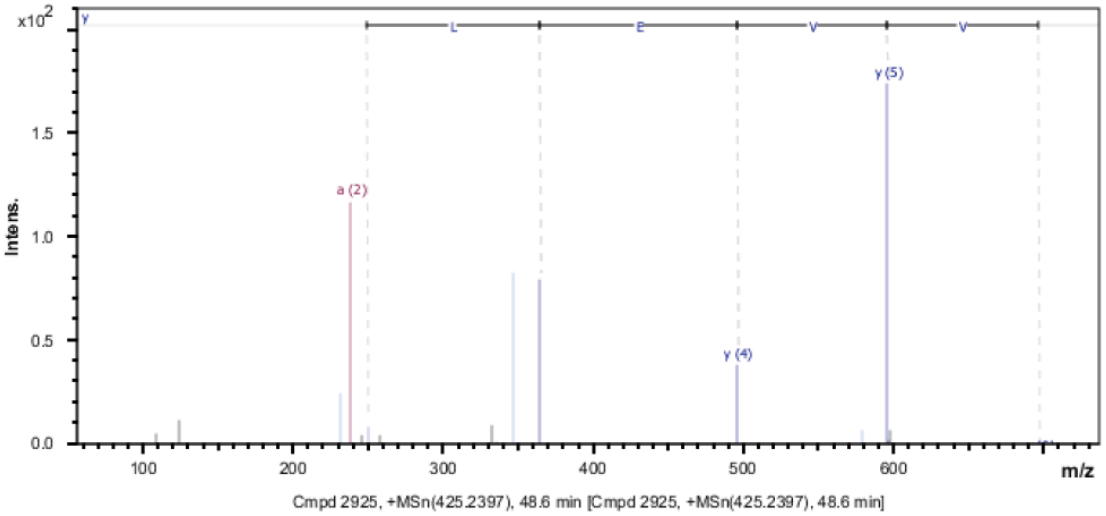
Protein Name: Os03g0713100 [Oryza sativa Japonica Group]
Accession: gi|297601571 **Parent m/z:** 568.93988, 3+
Sequence: K.TEGGQEYAEFLHAPR.K
Score: 44.97



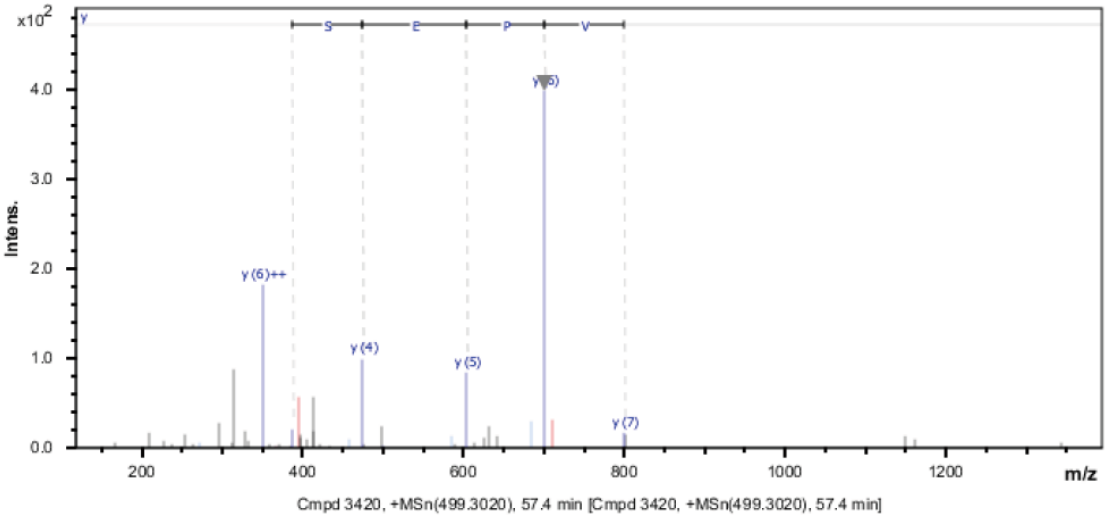
Protein Name: OSJNBa0027G07.8 [Oryza sativa (japonica cultivar-group)]
Accession: gi|32489187 **Parent m/z:** 695.671936, 3+
Sequence: K.GVDVDAEFNDILAAVEHDR.R
Score: 47.4



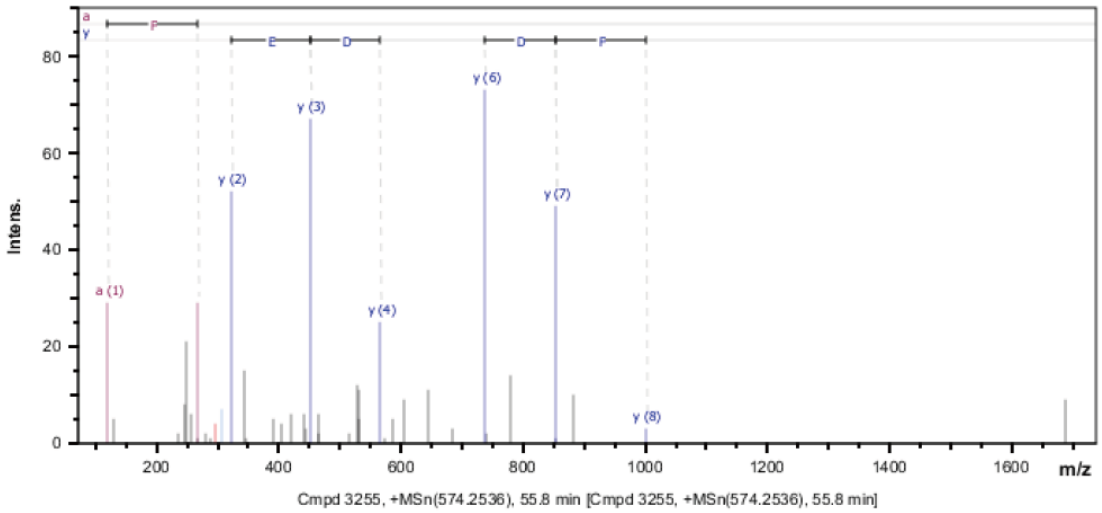
Protein Name: putative sucrosephosphate synthase [Oryza sativa Japonica Group]
Accession: gi|34015340 **Parent m/z:** 425.244385, 2+
Sequence: K.YVVELAR.A
Score: 32.92



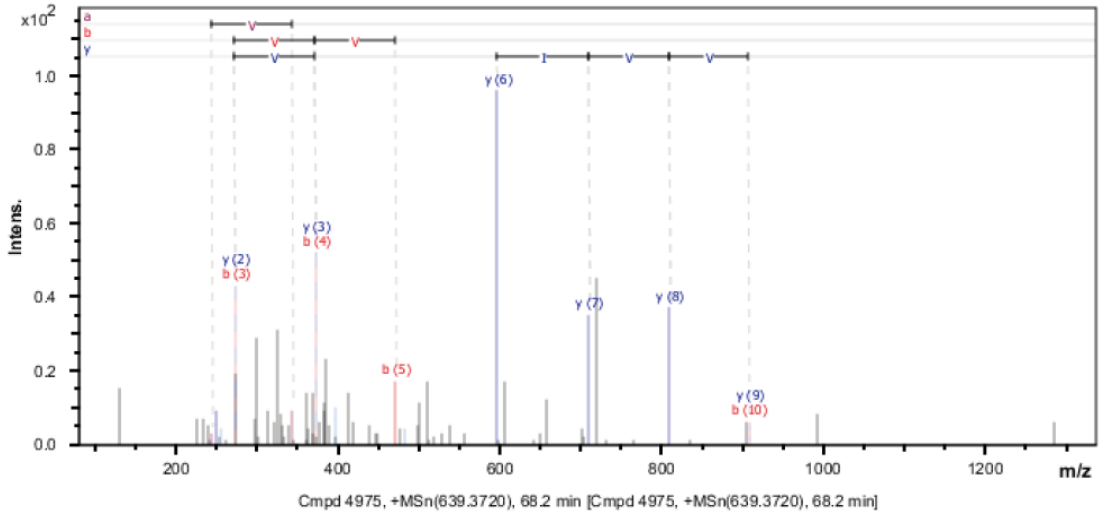
Protein Name: OSJNBa0035M09.3 [Oryza sativa (japonica cultivar-group)]
Accession: gi|38346542 **Parent m/z:** 499.300293, 2+
Sequence: K.VVVPESVLR.K
Score: 35.44



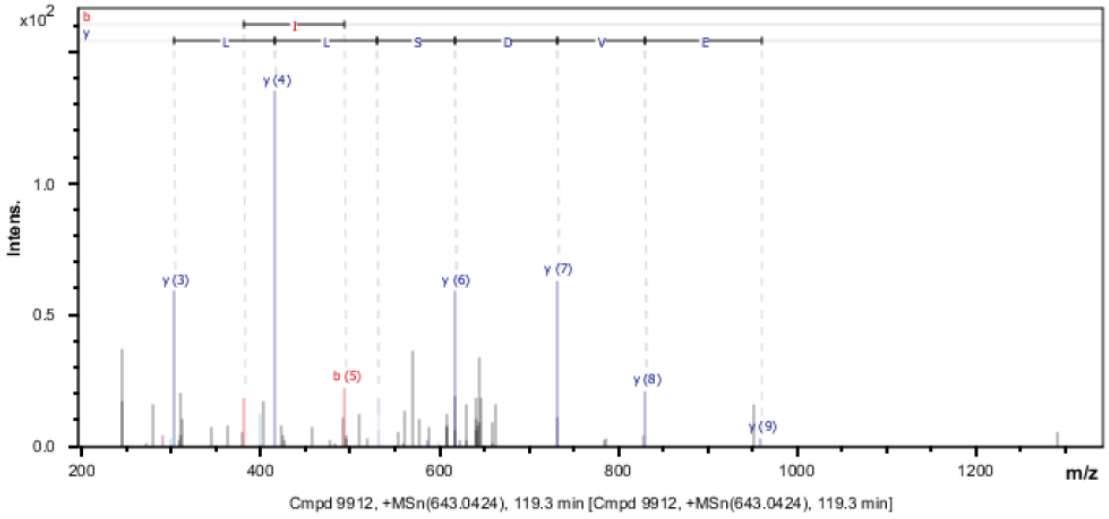
Protein Name: unknown protein [Oryza sativa Japonica Group]
Accession: gi|41052907 **Parent m/z:** 574.250366, 2+
Sequence: R.FFDGDDEFR.N
Score: 48.15



Protein Name: putative NAD-malate dehydrogenase [Oryza sativa Japonica Group]
Accession: gi|42407501 **Parent m/z:** 639.377258, 2+
Sequence: K.GVDVVVIPAGVPR.K
Score: 29.9

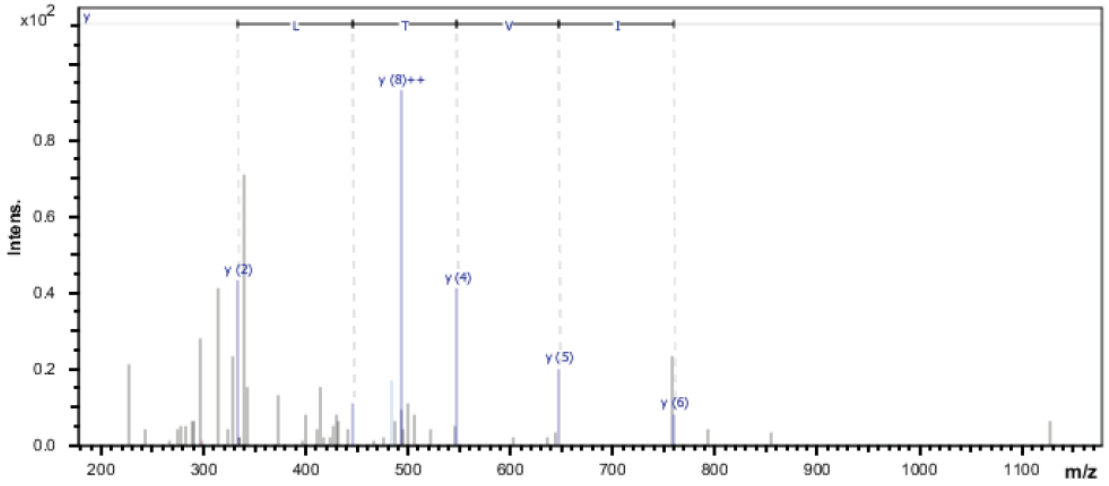


Protein Name: transitional endoplasmic reticulum ATPase-like [Oryza sativa Japonica Group]
Accession: gi|46390947 **Parent m/z:** 643.036499, 3+
Sequence: R.LAPVIIFVDEVDSLLGAR.G
Score: 43.88



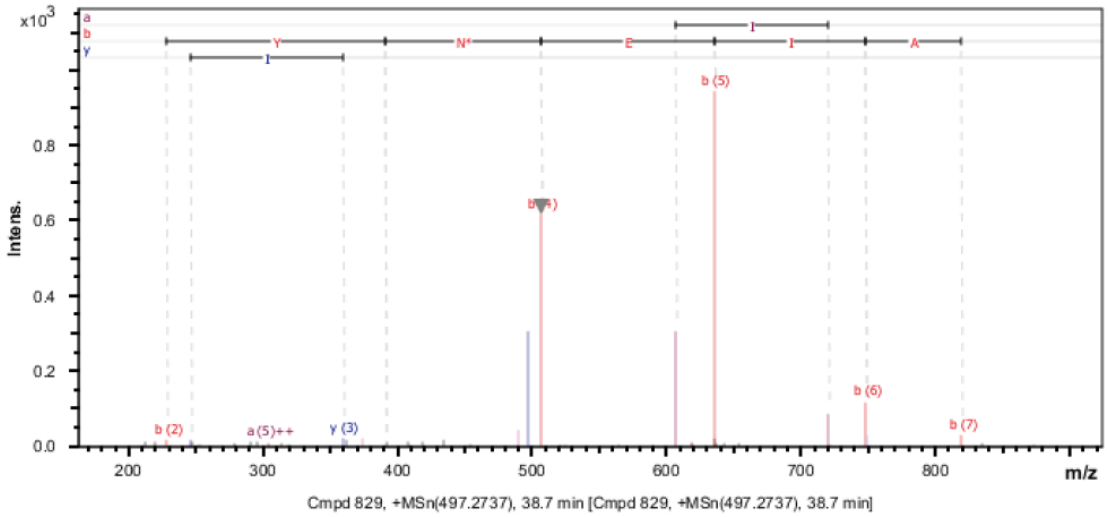
Cmpd 9912, +MSn(643.0424), 119.3 min [Cmpd 9912, +MSn(643.0424), 119.3 min]

Protein Name: putative Golgi-associated particle 102K chain [Oryza sativa Japonica Group]
Accession: gi|49387913 **Parent m/z:** 542.821838, 2+
Sequence: K.VPEIVTLWK.K
Score: 22.01



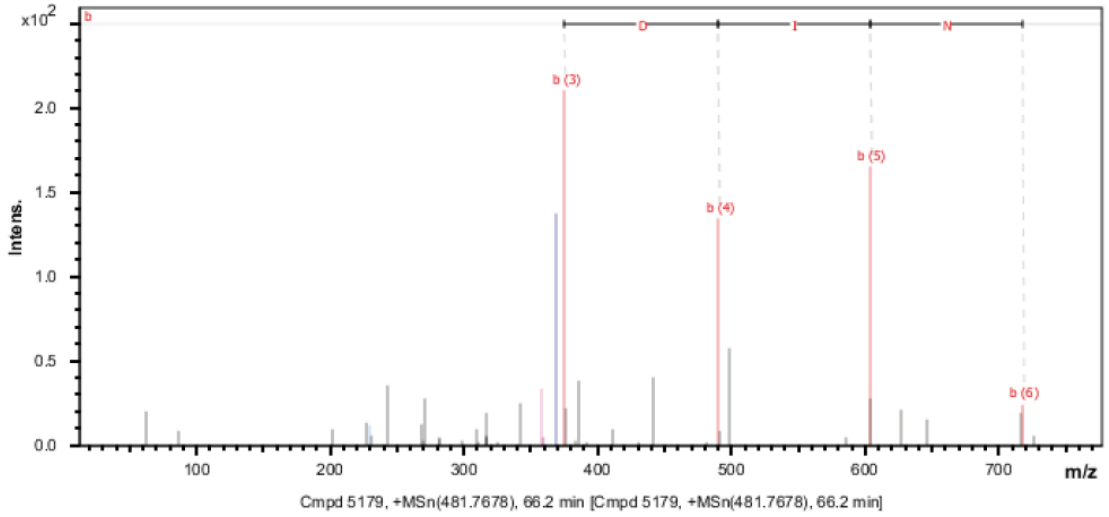
Cmpd 6878, +MSn(542.8278), 77.0 min [Cmpd 6878, +MSn(542.8278), 77.0 min]

Protein Name: hypothetical protein [Oryza sativa Japonica Group]
Accession: gi|50725842 **Parent m/z:** 497.271637, 2+
Sequence: R.NLYNEIAR.S **Modification:** Deamidated: 4
Score: 48.12



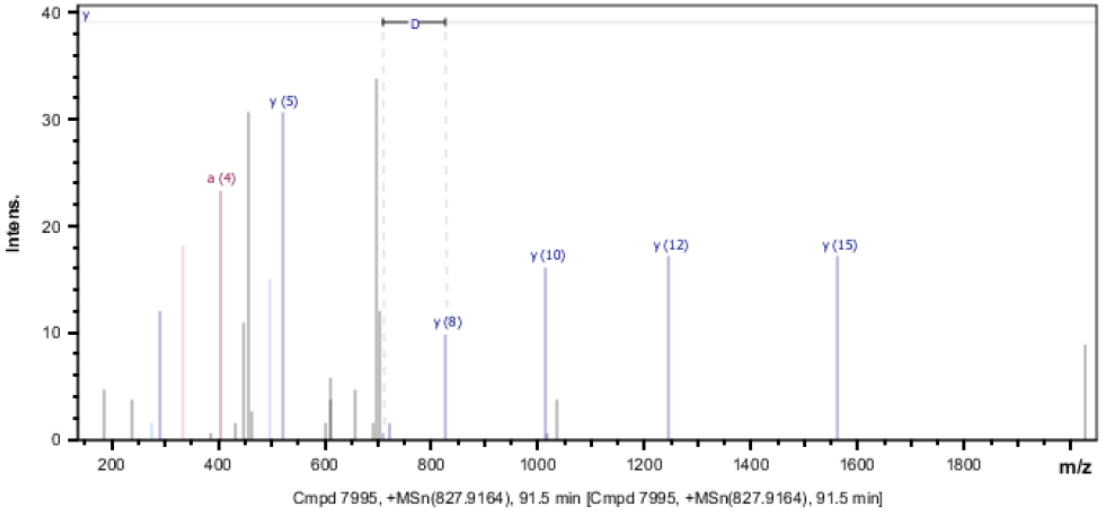
Cmpd 829, +MSn(497.2737), 38.7 min [Cmpd 829, +MSn(497.2737), 38.7 min]

Protein Name: ubiquitin conjugating enzyme-like [Oryza sativa Japonica Group]
Accession: gi|53791548 **Parent m/z:** 481.762817, 2+
Sequence: R.QVFDINAR.S
Score: 30.53

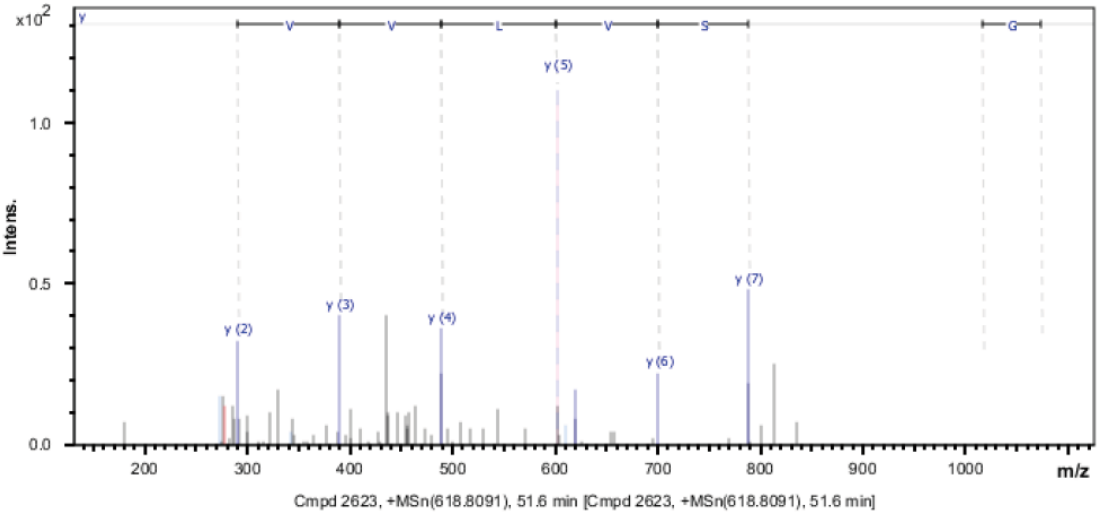


Cmpd 5179, +MSn(481.7678), 66.2 min [Cmpd 5179, +MSn(481.7678), 66.2 min]

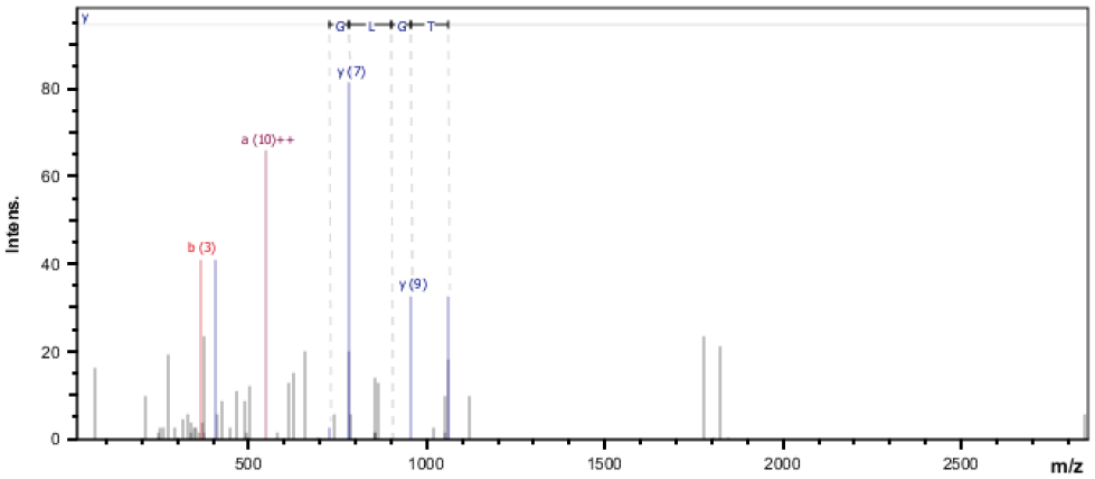
Protein Name: glycosylation enzyme-like [Oryza sativa Japonica Group]
Accession: gi|56202116 **Parent m/z:** 827.916016, 2+
Sequence: R.DDPVLDAIDADLLGGR.G
Score: 31.87



Protein Name: putative NtEPc-like protein [Oryza sativa Japonica Group]
Accession: gi|56783790 **Parent m/z:** 618.81311, 2+
Sequence: R.YGGGDSVLVVDR.E
Score: 73.5

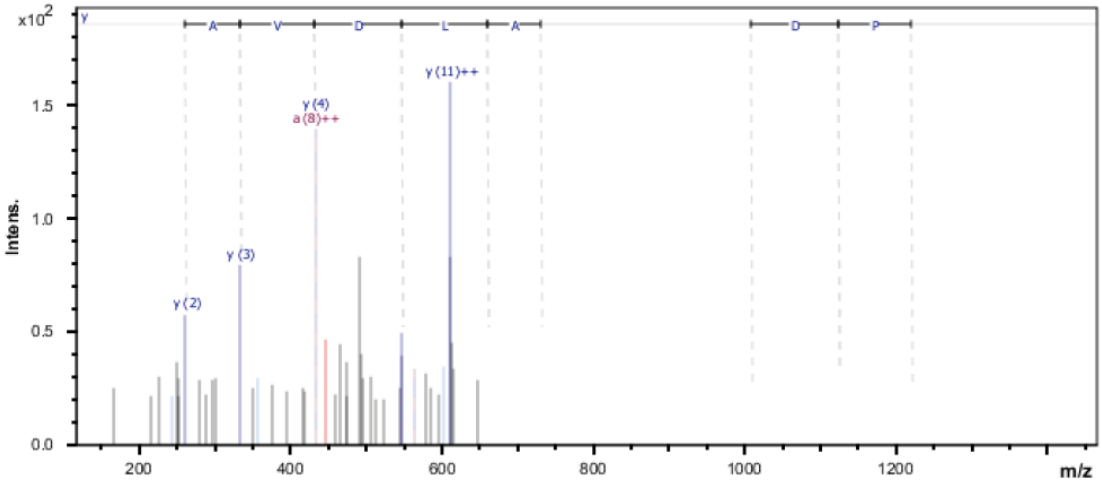


Protein Name: hypothetical protein LOC_Os11g22350 [Oryza sativa Japonica Group]
Accession: gi|62701757 **Parent m/z:** 855.447937, 2+
Sequence: K.VEEYFTGLGITVPER.V
Score: 22.36



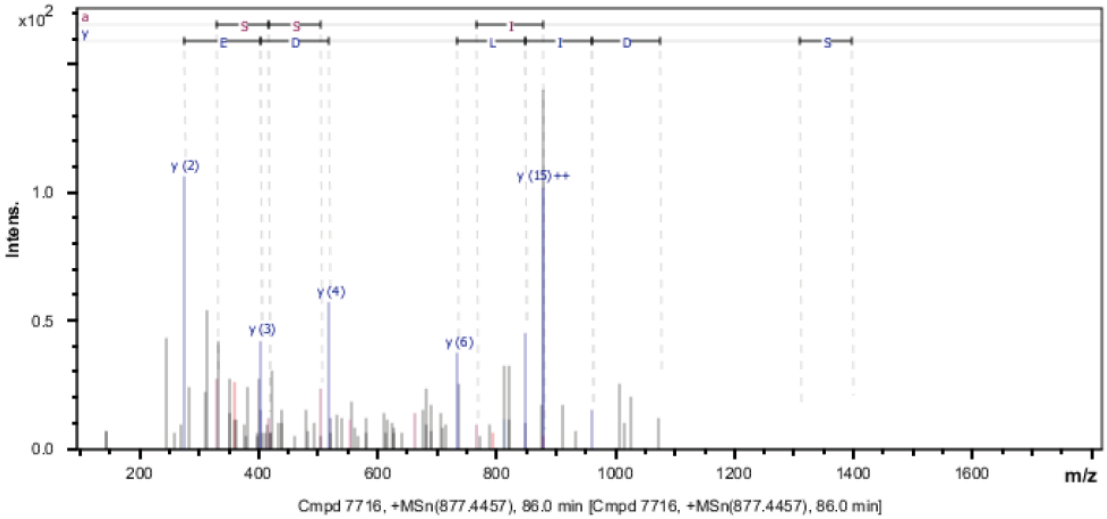
Cmpd 5791, +MSn(855.4486), 75.3 min [Cmpd 5791, +MSn(855.4486), 75.3 min]

Protein Name: OSJNBa0029H02.21 [Oryza sativa (japonica cultivar-group)]
Accession: gi|70663909 **Parent m/z:** 717.87195, 2+
Sequence: K.LTPDYDALDVANK.I
Score: 52.69



Cmpd 3761, +MSn(717.8719), 55.4 min [Cmpd 3761, +MSn(717.8719), 55.4 min]

Protein Name: glutaredoxin-related protein, expressed [Oryza sativa (japonica cultivar-group)]
Accession: gi|78708863 **Parent m/z:** 877.430908, 2+
Sequence: K.IPFSSFDILTDDEVR.Q
Score: 52.44



Protein Name: subtilisin-like serine protease [Oryza sativa Japonica Group]
Accession: gi|9453867 **Parent m/z:** 666.04126, 3+
Sequence: K.SHILPVAQIVYLSGEELK.A
Score: 53.86

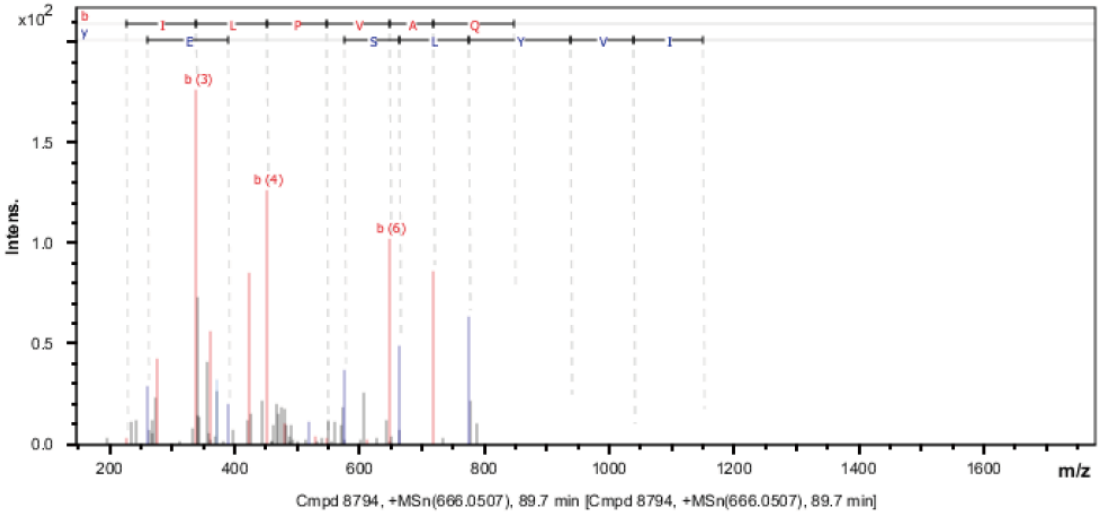


Figure S7. MS/MS fragmentation spectra of peptides, which led to identification of a protein by only itself.

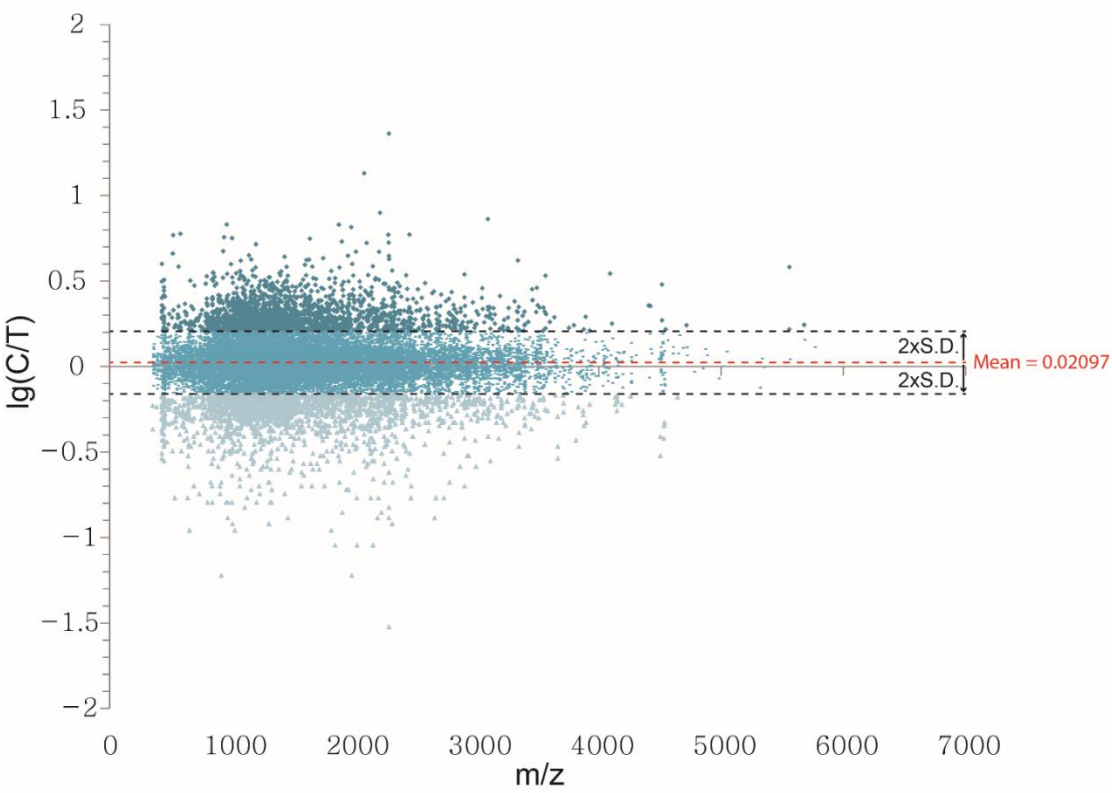


Figure S8. Volcano plot depicting the quantification results of label-free peptides. The red dotted line indicates the mean of lg(C/T); the black dotted line indicates 2 times the standard deviation (S.D.) of the lg(C/T) distance from the mean value. The calculation was based on RMS90 (root mean square, 90 percent confidence) peptides.

Table S3. Statistical analysis of gold particle distribution in developing rice pollen. A. Statistical analysis data from 5 independent experiments showing that lipid raft exist in rice pollen mother cells. B. Statistical analysis data from 5 independent experiments showing that lipid rafts migrate from vesicles to the plasma membrane as pollen develop. Duncan grouping shows significant analysis.

A

	Total number of gold particles			Number of clustered gold particles			Number of scattered gold particles		
	Mean ± S.D.	Duncan grouping		Mean ± S.D.	Duncan grouping		Mean ± S.D.	Duncan grouping	
		α = 0.05	α = 0.01		α = 0.05	α = 0.01		α = 0.05	α = 0.01
Mature Pollen (n=5)	167 ± 17.85	a	A	117 ±10.83	a	A	50 ± 7.37	a	A
Tetrad (n=5)	113 ± 8.88	b	B	67± 5.86	b	B	46 ± 5.29	ab	AB
Bicellular pollen (n=5)	88 ± 8.53	c	C	47 ±4.74	c	C	41 ± 5.68	bc	BC
Microspore (n=5)	60 ± 2.86	d	D	24 ±2.70	d	D	36 ± 1.30	c	C
Pollen mother cell (n=5)	20 ± 3.78	e	E	3 ± 0.45	e	E	17 ± 3.51	d	D

B

	Total number of clustered gold particles			Number of clustered gold particles in plasma membrane			Number of clustered gold particles in vesicles		
	Mean ± S.D.	Duncan grouping		Mean ± S.D.	Duncan grouping		Mean ± S.D.	Duncan grouping	
		α = 0.05	α = 0.01		α = 0.05	α = 0.01		α = 0.05	α = 0.01
Mature Pollen (n=5)	117 ± 10.83	a	A	77 ± 10.94	a	A	40 ± 3.54	a	A
Tetrad (n=5)	67 ± 5.86	b	B	28 ± 3.19	b	B	39 ± 4.91	a	A
Bicellular pollen (n=5)	47 ± 4.74	c	C	9 ± 2.68	c	C	38 ± 5.17	a	A
Microspore n=5)	24 ± 2.70	d	D	0	d	C	24 ± 2.70	b	B
Pollen mother cell (n=5)	3 ± 0.45	e	E	0	d	C	3 ± 0.45	c	C