

Supplementary Methods

Statistical analysis of 2-DE gels

Changes in protein abundance were compared for 2-DE gels generated from each strain using the program PD-Quest (Bio-Rad). Since the x,y -coordinates of spots on 2-DE gels from different bacterial isolates are not always identical due to minor amino acid sequence variations that lead to altered electrophoretic migration, and due to the nature of *P. aeruginosa* secreted protein gels containing large numbers of spots representing cleaved products from larger native proteins, we undertook a protein mapping exercise to identify like proteins across isolates, as well as image-based comparisons. Spots between isolates corresponding to the same protein identifications were detected using PD-Quest and the relative spot intensities (in ppm) calculated. Statistical analyses were performed on six replicate 2-DE gels corresponding to two gels from each of three separate biological preparations. Mean spot density values were calculated for each spot across replicate gels and standard error of the mean (SEM) determined. Spots absent from a given strain were denoted as not detected (-), while those only present in that strain were labeled (+). Students' t-test was performed on the normalized spot intensities, with significance levels set at 0.05.

Identification of 2-DE gel separated proteins by MALDI-TOF MS

Spots were destained in a 60:40 solution of 40 mM NH_4HCO_3 (pH 7.8)/100% acetonitrile (MeCN) for 1 h. Gel pieces were vacuum-dried for 1 h and rehydrated in 8 μL of 12 ng/ μL of trypsin at 4°C for 1 h. Excess trypsin was removed and gel pieces re-suspended in 25 μL of 40 mM NH_4HCO_3 and incubated overnight at 37°C. Peptides were concentrated and desalted using C18 Zip-Tips (Millipore, Bedford MA) and eluted in matrix (α -cyano-4-hydroxy cinnamic acid (Sigma), 8 mg/mL in 70% [v/v] MeCN/1% [v/v] formic acid [FA]) directly onto a target plate. Peptide mass maps were generated by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) using a QSTAR Elite mass spectrometer (Applied Biosystems, Foster City CA) equipped with o-MALDI source. Spectra were examined in Analyst v2.0 (Applied Biosystems) and mass calibration performed prior to data acquisition using external calibration with the Sequazyme™ peptide mass standard kit (Applied Biosystems). Peak

lists from each spot were generated by manual interrogation of the spectra.

Identification parameters included peptide mass accuracy within 0.08 Da, one possible missed tryptic cleavage per peptide and with the methionine sulfoxide and cysteine-acrylamide modifications checked. Identifications were based on MASCOT score, E-value at the 95% or > confidence level, observed pI and mass (kDa), number of matching peptide masses and total percentage of the amino acid sequence that those peptides covered.

2DLC-MS/MS of secreted proteins from *P. aeruginosa*

Dried secreted proteins (500 µg) from *P. aeruginosa* PAO1 and AES-1R grown in LB, M9 or ASMDM were resuspended in 6 M urea, 2 M thiourea, 40 mM NH₄HCO₃. Proteins were reduced for 1 hr with 10 mM dithiothreitol (DTT) followed by alkylation using 20 mM iodoacetamide for 1 hr in the dark. Alkylation was quenched using 10 mM DTT. 1/1000 (w/w) of endoproteinase Lys-C (WAKO Chemicals, Richmond VA) was added and digestion allowed to proceed for 4 hr at 25°C. Samples were diluted 1:5 with 100 mM NH₄HCO₃ and digested with 1/25 (w/w) of porcine modified sequencing grade trypsin (Promega, Madison WI) overnight at 25°C. 0.1% trifluoroacetic acid (TFA) of equal volume to the digested sample was added prior to solid phase extraction using Oasis® hydrophilic-lipophilic balance (HLB) extraction cartridges (Waters Corp., Milford MA). Acidified samples were applied to each HLB cartridge 5 times, and then washed with 5 mL 0.1% TFA. Bound peptides were eluted with 80% MeCN and lyophilized. Peptide fractionation was performed by offline strong cation exchange (SCX). Peptides were resuspended in loading buffer (30% (v/v) MeCN, 1% (v/v) acetic acid, pH 2.7) and applied to an SCX cartridge (PerSeptive Biosystems, Framingham MA). The column was washed in loading buffer and peptides eluted using 16 elution buffer steps (corresponding to 50, 110, 170, 230, 290, 350, 410, 470, 530, 590, 650, 710, 770, 830, 890, 950, 1000mM of KCl in loading buffer). The resulting fractions were desalted using POROS Oligo R3 (PerSeptive Biosystems) micro-columns. Peptides were eluted with 50% MeCN, 0.2% formic acid (FA), lyophilized, resuspended in 0.1% FA and then further separated on a trapless EASY-nano LC system (Proxeon, Odense Denmark). Peptides were loaded directly onto a 20 cm 100 µm inner diameter, 360 µm outer diameter, ReproSil – Pur C₁₈ AQ 3 µm (Dr. Maisch, Ammerbuch-Entringen,

Germany) reversed phase capillary column. The peptides were eluted using a gradient from 100% Buffer A (0.5% acetic acid) to 40% Buffer B (0.5% acetic acid, 80% MeCN) over 78 minutes at 200 nL / min directly into an LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, San Jose CA). The instrument was operated in a data-dependent mode automatically selecting the top 5 most intense ions with a charge state of +2 or greater for MS/MS. The selected ions were subjected to CID fragmentation with normalised collision energy of 35 and with dynamic exclusion enabled and set to 45 secs.

Supplementary Table S1. *P. aeruginosa* isolates used in this study.

Isolate	Genotype	Source	Reference
AES-1R	AES-1	CF epidemic isolates, Eastern Australia	Fung <i>et al</i> 2010, Hare <i>et al</i> 2012
AES-1M	AES-1		Naughton <i>et al</i> 2011
10-334A	AES-1		Manos <i>et al</i> 2008
AD-early	AES-1		Harmer <i>et al</i> 2012
AD-late	AES-1		Harmer <i>et al</i> 2012
CJ-early	AES-1		Harmer <i>et al</i> 2012
CJ-late	AES-1		Harmer <i>et al</i> 2012
DC-1A	AES-1		Harmer <i>et al</i> 2012
8Mel	AES-1		
LES431	Liverpool	CF epidemic isolates, Liverpool (LES)	Salunkhe <i>et al</i> 2005
LESB58	Liverpool		
Man C3373	Manchester	CF epidemic isolates, various centres in England and Wales	Scott <i>et al</i> 2004
Man 8799	Manchester		Scott <i>et al</i> 2004
Mid 10066	Midlands 1		Scott <i>et al</i> 2004
Mid 8916	Midlands 1		Scott <i>et al</i> 2004
Trent			
Stoke			
Leiden		CF epidemic isolate, The Netherlands	
Clone C			Römling <i>et al</i> 1994
17-early	Unique / Non-clonal	Non-epidemic CF isolates, Eastern Australia	Harmer <i>et al</i> 2012
17-late	Unique / Non-clonal *		Harmer <i>et al</i> 2012
9-early	Unique / Non-clonal		Harmer <i>et al</i> 2012
9-late	Unique / Non-clonal *		Harmer <i>et al</i> 2012
BB-early	Unique / Non-clonal		Harmer <i>et al</i> 2012
BB-late	Unique / Non-clonal *		Harmer <i>et al</i> 2012
NE43	Unique / Non-clonal	Non-LES epidemic CF isolates, Liverpool	Parsons <i>et al</i> 2002
NE127	Unique / Non-clonal		Parsons <i>et al</i> 2002
PAO1	Non-CF		ATCC

Fung, C.; *et al.*, *J. Med. Microbiol.* **2010**, 59 (9), 1089-1100.

Hare, N. J.; *et al.*, *BMC Microbiol.* **2012**, 12, 16.

Naughton, S.; *et al.*, *PLoS ONE* **2011**, 6 (9), e24526.

Manos, J.; *et al.*, *J. Med. Microbiol.* **2008**, 57 (12), 1454-1465.

Harmer, C. J.; *et al.*, *Microb. Pathog.* **2012**, 53 (1), 37-43.

Salunkhe, P.; *et al.*, *J. Bacteriol.* **2005**, 187 (14), 4908-4920.

Scott, F. W.; Pitt, T. L., *J. Med. Microbiol.* **2004**, 53 (7), 609-615.

Römling, U.; *et al.*, *J. Infect. Dis.* **1994**, 170 (6), 1616-1621.

Parsons, Y. N.; *et al.*, *J. Clin. Microbiol.* **2002**, 40 (12), 4607-4611.

Supplementary Table S2. Primers for quantitative real-time PCR and genomic screening.

Ac. No.	Gene	Forward primer (5' -> 3')	Reverse primer (5' -> 3')	Product size (bp)	Product T _m (°C)	Application
PA0423	<i>pasP</i>	TGACCATCAACACCAACAGC	CTTCGGTGGATTCTGAAGGTA	117	81.3	RT-PCR
PA1249	<i>aprA</i>	ACGGTGTACGGCTTCAACTC	AGAAGTCCAGGGTGTCGTTG	115	82.5	RT-PCR
	<i>16SrRNA</i>	AAGCAACGCGAAGAACCTTA	ATGCAGCACCTGTGTCTGAG	93	80.5	RT-PCR
AES_7139	<i>aes_7139</i>	GGAAGGTTTCGCCACAGAAT	CGGGTTGCCGTCTTTAGTTA	161	82.6	Screening
AES_7139	<i>aes_7139</i>	TATTCGCTATGCAAGGAGCA	TTCGTCCCAACAGAGAAACC	560	84.6	Screening
PA1777	<i>oprF</i>	GGTACTTCCTGACCGACGA	TCGCTGTTGATGTTGGTGAT	209	86.3	Screening

Supplementary Table S3. Identification of LB culture supernatant proteins from *P. aeruginosa* PAO1 and AES-1R. Proteins were separated by 2-DE (pH 4-7 and pH 6-11 gels) and identified by peptide mass mapping following trypsin digest and MASCOT search.

Spot No.	PA/AES Gene Name	Protein Identification	pI / Mass	MAS score	No. Match Peptides (No. Searched)	% Seq. Cov.
<i>P. aeruginosa</i> PAO1 (2-DE pH 4-7)						
1	PA1249	Alkaline Protease AprA	4.3 / 50.4	91	7 (13)	21%
2	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	184	13 (23)	28%
3-6*	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	191	15 (30)	32%
7	PA2300	Chitinase	5.2 / 53.0	119	9 (15)	25%
8	PA1092	Flagellin Type B FliC	5.4 / 49.2	145	12 (20)	38%
9-14	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	160	13 (24)	25%
15-16	PA1249	Alkaline Protease AprA	4.3 / 50.4	88	6 (8)	17%
18-20	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	90	9 (22)	19%
21	PA1249	Alkaline Protease AprA	4.3 / 50.4	88	6 (8)	17%
23-25	PA1092	Flagellin Type B FliC	5.4 / 49.2	128	10 (20)	29%
26	PA0572	Hypothetical Protein PA0572	6.1 / 100.1	100	11 (27)	13%
27-28	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	88	6 (9)	10%
32-33	PA1092	Flagellin Type B FliC	5.4 / 49.2	94	8 (11)	22%
34	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	86	6 (9)	12%
35	PA1092	Flagellin Type B FliC	5.4 / 49.2	88	6 (11)	18%
36-38	PA1092	Flagellin Type B FliC	5.4 / 49.2	154	10 (13)	22%
40	PA1092	Flagellin Type B FliC	5.4 / 49.2	111	7 (9)	21%
41	PA1249	Alkaline Protease AprA	4.3 / 50.4	60	4 (5)	8%
42	PA1092	Flagellin Type B FliC	5.4 / 49.2	143	8 (8)	16%
43-44	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	120	7 (7)	10%
46-47	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	88	5 (7)	18%
48-53	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	104	6 (8)	23%
54-59	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	143	8 (10)	30%
60-61	PA2939	Probable Aminopeptidase PA2939	5.4 / 49.2	97	5 (5)	11%
62	PA1092	Flagellin Type B FliC	5.4 / 49.2	101	6 (6)	18%
63	PA1092	Flagellin Type B FliC	5.4 / 49.2	114	10 (13)	22%
64	PA1092	Flagellin Type B FliC	5.4 / 49.2	110	8 (10)	20%
66	PA1094	Flagellar Capping Protein FliD	6.5 / 49.4	113	8 (13)	25%
67	PA1092	Flagellin Type B FliC	5.4 / 49.2	101	7 (9)	18%
69-73	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	117	6 (6)	18%
74-75	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	113	7 (7)	13%
76-84	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	66	5 (6)	8%
87	PA1092	Flagellin Type B FliC	5.4 / 49.2	83	5 (5)	14%
88	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	81	5 (5)	10%
89-90	PA1094	Flagellar Capping Protein FliD	6.5 / 49.4	78	6 (12)	18%
95	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
97	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
99	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
103	PA1092	Flagellin Type B FliC	5.4 / 49.2	48	3 (5)	12%
104	PA1094	Flagellar Capping Protein FliD	6.5 / 49.4	71	5 (8)	16%
105	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
106	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
107	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
108	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
109	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
110-111	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	4 (5)	10%
112	PA1092	Flagellin Type B FliC	5.4 / 49.2	74	5 (9)	17%
114	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	54	4 (5)	9%
115	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	54	4 (5)	9%
<i>P. aeruginosa</i> PAO1 (2-DE pH 6-11)						
1-2	PA0572	Hypothetical Protein PA0572	6.1 / 100.1	81	8 (11)	11%
3-5	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	123	14 (29)	31%
6	PA1572	Hypothetical Protein PA1572	5.2 / 40.7	64	7 (13)	24%
7	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	121	11 (20)	18%
8	PA1572	Hypothetical Protein PA1572	5.2 / 40.7	64	7 (13)	24%
9	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	60	7 (13)	15%

10	PA0572	Hypothetical Protein PA0572	6.1 / 100.1	73	8 (12)	11%
11	PA3922	Conserved Hypothetical Protein PA3922	8.6 / 51.2	72	8 (21)	25%
12	PA0572	Hypothetical Protein PA0572	6.1 / 100.1	73	8 (12)	11%
13	PA0572	Hypothetical Protein PA0572	6.1 / 100.1	64	7 (13)	10%
14	PA0852	Chitin-Binding Protein CbpD	6.4 / 41.9	78	5 (6)	19%
15-20	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	165	18 (28)	41%
21-22	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	131	12 (20)	30%
23	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	104	7 (10)	19%
24	PA3734	Hypothetical Protein PA3734	8.3 / 42.7	68	6 (12)	17%
25	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	104	7 (10)	19%
26-28	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	98	7 (11)	17%
29	PA1342	Probable Binding Protein Component of ABC Transporter	8.3 / 33.0	56	5 (12)	21%
30-33	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	110	6 (8)	16%
34-37	PA0852	Chitin-Binding Protein CbpD	6.4 / 41.9	63	7 (14)	17%
38	PA1136	Probable Transcriptional Regulator	5.7 / 27.1	55	6 (13)	23%
39-41	PA1871	LasA Protease	9.1 / 45.5	83	5 (6)	27%
42	PA0852	Chitin-Binding Protein CbpD	6.4 / 41.9	63	7 (14)	17%
43	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	65	5 (7)	11%
44	PA4232	Single-Stranded DNA Binding Protein	5.5 / 18.5	72	5 (11)	21%
45-47	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	61	5 (6)	9%

P. aeruginosa AES-1R (2-DE pH 4-7)

1-6	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	195	18 (33)	38%
7-9	AES3607	Chitinase PA2300	5.2 / 53.0	147	10 (12)	33%
10-	AES1954	Flagellin Type B PA1092	4.8 / 40.0	105	10 (26)	40%
11#	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	78	9 (26)	18%
12-15	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	186	16 (24)	37%
17	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	114	11 (22)	22%
18-19	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	125	11 (18)	22%
22	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	123	11 (19)	22%
23-24	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	116	10 (17)	19%
26	AES3607	Chitinase PA2300	5.2 / 53.0	99	9 (18)	27%
28-29	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	120	10 (15)	19%
30	AES1954	Flagellin Type B FlhC PA1092	4.8 / 40.0	81	8 (12)	29%
31-38	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	130	12 (22)	25%
39-40	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	113	10 (18)	19%
41	AES1954	Flagellin Type B FlhC PA1092	4.8 / 40.0	62	6 (15)	20%
42-47	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	96	8 (13)	22%
48	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	82	7 (13)	19%
52-54	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	90	8 (14)	17%
55	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	66	7 (13)	13%
57-58	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	81	7 (12)	16%
61-62	AES0809	PasP Protease PA0423	6.1 / 20.8	146	13 (25)	65%
63-64	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	81	7 (12)	16%
69-70	AES1561	Chitin-Binding Protein CbpD PA0852	6.4 / 41.9	70	5 (8)	23%
73-75	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	81	5 (5)	10%
91	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	81	7 (12)	16%
92	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	81	7 (12)	16%
94-95	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	65	5 (6)	9%

P. aeruginosa AES-1R (2-DE pH 6-11)

1	AES0579	Hypothetical Protein PA0572	6.1 / 100.1	138	14 (20)	20%
2-3	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	98	11 (30)	30%
4-6	AES2685	Lipoamide Dehydrogenase-Glc PA1587	6.5 / 50.1	138	14 (26)	34%
7	AES1611	Conserved Hypothetical Protein PA3922	8.6 / 51.2	84	8 (17)	20%
8	AES1611	Conserved Hypothetical Protein PA3922	8.6 / 51.2	172	18 (34)	46%
9	AES0565	Sulfate-Binding Protein of ABC Transporter PA1493	7.9 / 36.5	70	8 (21)	37%
10	AES1561	Chitin-Binding Protein CbpD PA0852	6.4 / 41.9	100	11 (22)	43%
11	AES0417	Polyamine Transport Protein PA0300	7.0 / 40.6	64	9 (23)	33%
12#	AES1561	Chitin-Binding Protein CbpD PA0852	6.4 / 41.9	72	9 (36)	38%
	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	60	9 (36)	24%
13-16	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	139	17 (31)	33%
17-18	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	152	20 (48)	33%
19	AES2411	Putative Binding Protein Component of ABC Transporter PA1342	9.4 / 29.5	116	12 (30)	46%
20-21	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	85	11 (30)	20%
23-25	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	75	9 (23)	22%

26-27	AES1561	Chitin-Binding Protein CbpD PA0852	6.4 / 41.9	41	6 (19)	22%
28-30	AES3084	LasA Protease PA1871	9.1 / 45.5	55	7 (33)	33%
31	AES6438	Conserved Hypothetical Protein PA5001	8.7 / 38.1	39	5 (24)	36%
	AES1561	Chitin-Binding Protein CbpD PA0852	6.4 / 41.9	32	5 (24)	12%
32	AES0680	Conserved Hypothetical Protein PA0329	8.9 / 12.4	69	5 (19)	36%
33	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	40	6 (22)	13%
35	AES6904	Putative ABC Transporter, Periplasmic Binding Protein PA5505	8.5 / 28.3	109	10 (33)	44%
36-38	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	57	7 (22)	18%
40	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	67	10 (38)	25%
	AES1561	Chitin-Binding Protein CbpD PA0852	6.4 / 41.9	57	8 (38)	32%
41	AES5204	Elastase LasB (Pseudolysin) PA3724	6.3 / 53.7	145	16 (35)	33%

Spot, refers to spot numbers shown on representative 2-DE gels shown in Figure 1; PA/AES Gene Name, gene assignment from ¹ and AES-1R genome sequence ³³; MAS, MASCOT score from data search; % Seq. Cov., the % of the sequence covered by those matches according to the MASCOT search. # more than 1 protein confidently identified in a single spot.

Supplementary Table S4. Identification of M9 culture supernatant proteins from *P. aeruginosa* PAO1 and AES-1R. Proteins were separated by 2-DE (pH 4-7 gels) and identified by peptide mass mapping following trypsin digest and MASCOT search.

Spot No.	PA/AES Gene Name	Protein Identification	pI / Mass	MAS score	No. Match Peptides (No. Searched)	% Seq. Cov.
<i>P. aeruginosa</i> PAO1						
1-3	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	125	8 (24)	26%
4	PA1092	Flagellin Type B FliC	5.4 / 49.2	166	13 (17)	34%
5	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	99	10 (17)	19%
6	PA1086	Flagellar Hook-Associated Protein 1 FlgK	4.6 / 71.7	132	14 (17)	26%
7	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	102	9 (14)	15%
8	PA2939	Probable Aminopeptidase PA2939	5.0 / 57.5	59	5 (8)	10%
9	PA1092	Flagellin Type B FliC	5.4 / 49.2	122	11 (19)	32%
10	PA0572	Hypothetical Protein	6.12 / 100.1	123	15 (27)	19%
11-13	PA1092	Flagellin Type B FliC	5.4 / 49.2	120	10 (15)	28%
14	PA1092	Flagellin Type B FliC	5.4 / 49.2	114	11 (21)	28%
15	PA3724	Elastase LasB (Pseudolysin)	5.8 / 32.9	136	12 (27)	47%
16	PA1086	Flagellar Hook-Associated Protein 1 FlgK	4.6 / 71.7	51	6 (15)	15%
17-18	PA1092	Flagellin Type B FliC	5.4 / 49.2	101	9 (15)	23%
19	PA1086	Flagellar Hook-Associated Protein 1 FlgK	4.6 / 71.7	82	9 (18)	17%
20-25	PA1092	Flagellin Type B FliC	5.4 / 49.2	114	11 (20)	29%
26	PA2451	Hypothetical Protein	5.0 / 22.0	62	5 (15)	37%
27	PA1094	Flagellar Capping Protein FliD	6.5 / 49.4	57	5 (10)	16%
28#	PA1092	Flagellin Type B FliC	5.4 / 49.2	76	8 (19)	25%
	PA1094	Flagellar Capping Protein FliD	6.5 / 49.4	67	7 (19)	24%
29	PA1092	Flagellin Type B FliC	5.4 / 49.2	111	9 (13)	26%
30	PA0572	Hypothetical Protein	6.12 / 100.1	57	6 (10)	8%
32	PA0852	Chitin-Binding Protein CbpD	6.4 / 41.9	61	5 (14)	21%
33-38	PA3407	Heme Acquisition Protein HasAp	4.3 / 20.9	48	4 (10)	44%
39	PA0423	PasP Protease PA0423	6.4 / 20.8	103	8 (21)	50%
40-44	PA1094	Flagellar Capping Protein FliD	6.5 / 49.4	101	8 (11)	26%
45	PA1092	Flagellin Type B FliC	5.4 / 49.2	73	5 (6)	15%
49	PA2451	Hypothetical Protein	5.0 / 22.0	38	3 (9)	21%
51	PA1092	Flagellin Type B FliC	5.4 / 49.2	68	5 (7)	15%
53	PA2451	Hypothetical Protein	5.0 / 22.0	62	3 (3)	19%
<i>P. aeruginosa</i> AES-1R						
1-2	AES1954	Flagellin Type B FliC PA1092	4.8 / 40.0	198	14 (18)	46%
3-5	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	156	13 (19)	25%
6	AES1929	Branched-Chain Amino Acid Transport Protein BraC PA1074	5.6 / 39.7	59	6 (18)	29%
7	AES1954	Flagellin Type B FliC PA1092	4.8 / 40.0	74	6 (11)	23%
8	AES4696	Putative Binding Protein Component of ABC Sugar Transporter PA3190	5.7 / 45.1	106	10 (16)	25%
9	AES1954	Flagellin Type B FliC PA1092	4.8 / 40.0	82	6 (9)	23%
10	AES0668	Putative Glycine Betaine/L-Proline ABC Transporter Component PA5378	6.8 / 34.4	72	8 (31)	30%
11	AES1954	Flagellin Type B FliC PA1092	4.8 / 40.0	72	6 (10)	17%
12	AES6603	Putative ABC Transporter, Periplasmic Amino Acid-Binding Protein PA5153	5.1 / 27.5	113	9 (19)	44%
13	AES2249	Heme Acquisition Protein HasAp PA3407	4.3 / 20.9	38	2 (3)	24%
14	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	50	5 (19)	9%
15	AES5824	Superoxide Dismutase SodB PA4366	5.3 / 21.3	62	6 (16)	45%
16	AES0809	PasP Protease PA0423	6.1 / 20.8	114	9 (23)	48%
20	AES6767	Putative Endoribonuclease L-PSP PA5339	5.1 / 13.6	107	7 (17)	75%
22	AES1954	Flagellin Type B FliC PA1092	4.8 / 40.0	116	10 (19)	23%
25	AES1235	Putative Phage Tail Tube Protein PA0623	5.1 / 18.0	106	7 (13)	41%
27-28	AES3607	Chitinase PA2300	5.2 / 53.0	133	9 (11)	29%
29	AES5528	Inorganic Pyrophosphatase Ppa	5.0 / 19.4	57	4 (9)	32%
31	AES1929	Branched-Chain Amino Acid Transport Protein BraC PA1074	5.6 / 39.7	76	6 (11)	32%
32	AES4384	Putative Aminopeptidase PA2939	5.0 / 57.5	62	6 (12)	11%

33	AES1954	Flagellin Type B FliC PA1092	4.8 / 40.0	128	9 (12)	30%
34	AES0809	PasP Protease PA0423	6.1 / 20.8	143	11 (26)	48%
35	AES1650	Arginine/Ornithine Binding Protein AotJ PA0888	6.4 / 29.0	140	9 (11)	41%

Spot, refers to spot numbers shown on representative 2-DE gels shown in Figure 5.2; PA/AES Gene Name, gene assignment from ¹ and AES-1R genome sequence ³³; MAS, MASCOT score from data search; % Seq. Cov., the % of the sequence covered by those matches according to the MASCOT search.

Supplementary Table S5. Identification of LB culture supernatant proteins from *P. aeruginosa* PAO1 and AES-1R. Proteins were identified by trypsin digest and 2DLC-MS/MS.

PA/AES Gene Name	Protein Identification	MW (kDa)	PAO1				AES-1R			
			No. Unique Pep.	No. Unique Spectra	No. Total Spectra	Seq. Cov. (%)	No. Unique Peptides	No. Unique Spectra	No. Total Spectra	Seq. Cov. (%)
PA0852	Chitin-Binding Protein CbpD Precursor+	42	29	33	59	26.00%	38	39	53	46.00%
PA3724	Elastase LasB+	54	40	52	127	23.00%	35	47	103	22.00%
PA1587	Lipoamide Dehydrogenase-Glc	50	2	2	2	4.40%	17	17	21	26.00%
PA1342	Probable Binding Protein Component of ABC Transporter+	33	6	7	11	7.00%	12	14	23	26.00%
PA2939	Probable Aminopeptidase+	58	16	17	19	15.00%	11	11	14	13.00%
PA1092	Flagellin Type B+	40	23	25	39	4.30%	10	11	14	13.00%
PA0888	Arginine/Ornithine Binding Protein AotJ+	29	3	3	4	5.20%	10	10	13	22.00%
PA0895	Bifunctional Succinylornithine Transaminase/Acetylornithine Aminotransferase	44	3	3	4	9.90%	9	10	12	22.00%
PA1871	LasA Protease+	45	6	6	9	7.30%	7	8	13	12.00%
PA1074	Branched-Chain Amino Acid Transport Protein BraC+	40	4	6	7	10.00%	7	8	10	12.00%
PA0266	4-Aminobutyrate Aminotransferase	45	3	3	3	9.60%	7	7	8	12.00%
PA2250	Lipoamide Dehydrogenase-Val	49	3	3	4	3.40%	6	6	7	7.80%
PA0300	Polyamine Transport Protein+	42	2	2	2	4.00%	5	5	7	9.30%
PA0423	PasP Protease+	21	1	1	1	4.20%	5	5	6	17.00%
PA4922	Azurin Precursor+	16	1	1	2	6.80%	5	7	9	34.00%
PA0482	Malate Synthase G	79	4	4	4	5.00%	4	4	5	6.20%
PA2966	Acyl Carrier Protein	9	4	4	6	22.00%	4	4	7	22.00%
PA5489	Thiol:Disulfide Interchange Protein DsbA+	23	3	3	3	14.00%	4	4	5	15.00%
PA4266	Elongation Factor G	78	1	1	1	1.80%	4	4	4	5.10%
PA5171	Arginine Deiminase	46	1	1	1	3.80%	4	4	6	3.80%
PA4175	Pvds-Regulated Endoprotease, Lysyl Class PrpL+	47	4	4	4	9.60%	3	3	3	5.80%
PA5240	Thioredoxin	12	4	4	5	11.00%	3	3	4	19.00%
PA4761	Putative Heat Shock Protein	68	2	2	3	2.70%	3	3	3	3.90%
PA3083	Aminopeptidase N	100	1	1	1	2.00%	3	3	3	2.80%
PA4496	Probable Binding Protein Component of ABC Transporter+	60	1	1	1	2.60%	3	3	3	5.40%
PA0572	Hypothetical Protein+	100	8	8	9	4.10%	2	2	3	1.80%
PA4236	Catalase+	56	5	5	7	3.90%	2	2	2	3.30%
PA1337	Glutaminase-Asparaginase+	37	2	2	2	6.60%	2	2	3	6.90%
PA1777	Major Porin and Structural Outer Membrane Porin OprF Precursor+	38	2	2	2	3.70%	2	2	2	3.70%
PA1874	Hypothetical Protein	238	2	2	2	0.97%	2	2	2	0.73%
PA3807	Nucleoside Diphosphate Kinase	16	2	2	3	13.00%	2	2	6	13.00%
PA0026	Phospholipase C, PlcB+	37	1	1	1	2.70%	2	2	3	6.40%
PA2444	Serine Hydroxymethyltransferase	45	1	1	1	3.10%	2	2	3	9.10%
PA3621	Ferredoxin I	12	1	1	1	14.00%	2	2	2	15.00%
PA4366	Superoxide Dismutase+	21	1	1	1	4.70%	2	2	3	11.00%
PA5013	Branched-Chain Amino Acid Aminotransferase	34	1	1	1	2.90%	2	2	2	6.80%

PA1093	Hypothetical Protein	13	4	4	5	25.00%	1	1	1	7.80%
PA5285	Hypothetical Protein	11	3	3	3	38.00%	1	1	1	19.00%
PA1094	Flagellar Capping Protein Flid+	49	2	2	3	2.70%	1	1	1	4.00%
PA3309	Conserved Hypothetical Protein	16	2	2	2	9.10%	1	2	3	9.10%
PA4385	Chaperonin GroEL	57	2	2	2	2.40%	1	1	1	2.20%
PA5100	Urocanate Hydratase	61	2	2	2	2.70%	1	1	1	2.10%
PA1249	Alkaline Protease AprA +	50	19	19	23	15.00%	n.d.	n.d.	n.d.	n.d.
PA1784	Putative Lyase	27	2	2	3	6.40%	n.d.	n.d.	n.d.	n.d.
PA0139	Alkyl Hydroperoxide Reductase Subunit C	21	2	2	2	9.60%	n.d.	n.d.	n.d.	n.d.
PA5312	Putative Aldehyde Dehydrogenase	53	2	2	2	5.20%	n.d.	n.d.	n.d.	n.d.
PA3190	Probable Binding Protein Component of ABC Sugar Transporter+	45	n.d.	n.d.	n.d.	n.d.	9	10	11	17.00%
AES_7139	Hypothetical Protein	17	n.d.	n.d.	n.d.	n.d.	5	6	7	32.00%
PA0973	Peptidoglycan Associated Lipoprotein OprL Precursor+	18	n.d.	n.d.	n.d.	n.d.	5	5	7	18.00%
PA1293	Hypothetical Protein	39	n.d.	n.d.	n.d.	n.d.	5	5	7	10.00%
PA4847	Acetyl-CoA Carboxylase Biotin Carboxyl Carrier Protein Subunit	16	n.d.	n.d.	n.d.	n.d.	5	6	7	24.00%
PA0432	S-Adenosyl-L-Homocysteine Hydrolase	51	n.d.	n.d.	n.d.	n.d.	4	4	4	6.50%
PA0039	Conserved Hypothetical Protein	9	n.d.	n.d.	n.d.	n.d.	3	3	5	14.00%
PA0456	Putative Major Cold Shock Protein	8	n.d.	n.d.	n.d.	n.d.	3	3	3	20.00%
PA1493	Sulfate-Binding Protein of ABC Transporter+	36	n.d.	n.d.	n.d.	n.d.	3	3	3	8.70%
PA1689	Putative Membrane Protein	49	n.d.	n.d.	n.d.	n.d.	3	3	3	7.00%
PA2025	Glutathione Reductase	49	n.d.	n.d.	n.d.	n.d.	3	3	4	2.90%
PA5554	ATP Synthase Beta Chain	50	n.d.	n.d.	n.d.	n.d.	3	3	3	3.90%
PA1202	Probable Hydrolase	22	n.d.	n.d.	n.d.	n.d.	2	2	2	16.00%
PA3123	Putative Translation Initiation Inhibitor	14	n.d.	n.d.	n.d.	n.d.	2	2	2	19.00%
PA3922	Conserved Hypothetical Protein	51	n.d.	n.d.	n.d.	n.d.	2	2	2	3.10%
PA4015	Conserved Hypothetical Protein	17	n.d.	n.d.	n.d.	n.d.	2	2	2	11.00%
PA5046	Malic Enzyme	45	n.d.	n.d.	n.d.	n.d.	2	2	2	2.60%
PA5339	Putative Endoribonuclease L-PSP	14	n.d.	n.d.	n.d.	n.d.	2	2	2	13.00%

+ proteins denoted as non-cytoplasmic or extracellular from the PseudoCAP database (www.pseudomonas.com)

Supplementary Table S6. Identification of M9 culture supernatant proteins from *P. aeruginosa* PAO1 and AES-1R. Proteins were identified by trypsin digest and 2DLC-MS/MS.

Protein access. No.	Protein Name	MW (kDa)	PAO1				AES-1R			
			No. Unique Pep.	No. Unique Spectra	No. Total Spectra	Seq. Cov. (%)	No. Unique Pep.	No. Unique Spectra	No. Total Spectra	Seq. Cov. (%)
PA3190	Probable Binding Protein Component Of ABC Sugar Transporter+	45	20	20	24	44.0%	76	93	142	74.0%
PA1092	Flagellin Type B+	40	104	132	284	63.0%	71	95	196	81.0%
PA4175	Pvds-Regulated Endoprotease, Lysyl Class PrpL+	47	48	56	96	35.0%	69	77	135	58.0%
PA4761	Putative Heat Shock Protein	68	36	36	36	44.0%	59	72	105	57.0%
PA4265	Elongation Factor Tu	43	17	20	20	41.0%	57	68	105	70.0%
PA0852	Chitin-Binding Protein CbpD Precursor+	42	84	92	197	71.0%	48	53	89	68.0%
PA4385	Chaperonin GroEL	57	8	8	9	12.4%	44	47	61	48.0%
PA4266	Elongation Factor G	78	28	28	36	26.0%	36	38	47	44.0%
PA0482	Malate Synthase G	79	20	21	24	27.6%	34	34	42	46.0%
PA1342	Probable Binding Protein Component Of ABC Transporter+	33	40	40	44	49.0%	32	38	63	51.0%
PA5016	Dihydrolipoamide Acetyltransferase	57	4	4	4	6.4%	32	46	49	39.0%
PA2939	Probable Aminopeptidase+	58	24	24	28	37.0%	32	34	49	47.0%
PA3724	Elastase LasB+	54	56	61	116	56.0%	32	34	49	45.0%
PA5046	Malic Enzyme+	45	4	4	4	12.5%	31	34	40	73.0%
PA1587	Lipoamide Dehydrogenase-Glc	50	4	5	5	11.1%	31	32	49	46.0%
PA1074	Branched-Chain Amino Acid Transport Protein BraC+	40	12	20	24	23.6%	29	38	54	57.0%
PA3162	30S Ribosomal Protein S1	62	12	12	12	24.8%	29	29	42	32.0%
PA1596	Heat Shock Protein HtpG	72	4	4	4	7.6%	27	28	37	33.0%
PA3407	Heme Acquisition Protein HasAp+	21	32	48	145	76.0%	27	32	58	73.0%
PA1871	Staphylolytic Protease Preproenzyme LasA+	45	4	4	4	8.8%	26	26	33	31.0%
PA1588	Succinyl-CoA Synthetase Beta Chain	42	16	16	23	36.0%	25	25	30	38.0%
PA4922	Azurin Precursor+	16	17	24	28	56.0%	25	36	70	68.0%
PA0423	Putative Periplasmic Protein+	21	17	19	26	59.0%	23	30	52	63.0%
PA3397	Ferredoxin--NADP+ Reductase	30	32	35	40	68.0%	23	26	37	70.0%
PA0300	Polyamine Transport Protein+	42	7	8	8	16.0%	23	25	33	57.0%
PA3635	Phosphopyruvate Hydratase	45	8	9	9	16.8%	23	25	30	39.0%
PA5171	Arginine Deiminase	46	4	8	10	17.2%	23	25	30	59.0%
PA1094	Flagellar Capping Protein Flid+	49	47	52	115	60.0%	23	25	40	40.0%
PA0139	Alkyl Hydroperoxide Reductase Subunit C	21	5	5	6	29.0%	21	25	37	68.0%
PA4602	Serine Hydroxymethyltransferase	45	4	4	5	7.6%	21	23	28	46.0%
PA1800	Trigger Factor	49	8	9	9	26.2%	21	21	28	28.0%
PA0588	Putative Protein Kinase	130	6	8	11	5.2%	21	21	26	12.0%
PA0122	Conserved Hypothetical Protein+	15	4	5	6	44.0%	21	23	47	86.0%
PA0623	Putative Phage Tail Tube Protein	18	4	4	5	38.0%	21	25	37	76.0%
PA0888	Arginine/Ornithine Binding Protein AotJ+	29	12	12	14	20.8%	21	23	30	45.0%
PA4496	Probable Binding Protein Component Of ABC Transporter+	60	4	4	5	12.0%	21	21	26	39.0%
PA1609	Beta-Ketoacyl-ACP Synthase I	43	12	12	16	26.8%	21	21	23	36.0%
PA4542	ClpB Protein	94	5	5	7	5.2%	19	19	23	16.6%
PA4370	Insulin-Cleaving Metalloproteinase Outer Membrane Protein Precursor+	47	12	12	13	26.0%	19	19	23	35.0%

PA3763	Phosphoribosylformylglycinamide Synthase	141	12	12	16	10.8%	19	19	21	14.0%
PA1086	Flagellar Hook-Associated Protein 1 FlgK+	72	48	48	48	72.0%	18	18	35	50.8%
PA3529	Putative Alkyl Hydroperoxide Reductase Subunit	22	4	4	4	18.0%	17	19	30	65.0%
PA0895	Bifunctional Succinylornithine Transaminase/Acetylornithine Aminotransferase	44	16	16	18	39.6%	17	19	23	45.0%
PA1787	Bifunctional Aconitate Hydratase 2/2-Methylisocitrate Dehydratase	94	20	20	23	21.2%	17	19	21	16.0%
PA1777	Major Porin And Structural Outer Membrane Porin OprF Precursor+	38	8	8	10	21.6%	17	19	26	38.0%
PA5153	Putative ABC Transporter, Periplasmic Amino Acid-Binding Protein+	28	7	10	10	38.4%	17	17	23	55.0%
PA5554	ATP Synthase Beta Chain	50	24	24	28	30.4%	17	17	19	28.0%
PA0459	Putative ClpA/B Protease ATP Binding Subunit	103	4	4	5	6.4%	15	17	21	14.0%
PA3807	Nucleoside Diphosphate Kinase	16	16	20	27	41.0%	15	19	28	43.0%
PA5489	Thiol:Disulfide Interchange Protein DsbA+	23	8	8	8	26.4%	15	17	21	46.0%
PA4739	Conserved Hypothetical Protein+	12	20	20	20	56.0%	13	13	26	53.0%
PA1087	Flagellar Hook-Associated Protein Type 3 FlgL+	47	32	32	40	42.0%	13	13	14	20.0%
PA2462	Hypothetical Protein+	532	132	136	151	22.8%	13	13	14	2.9%
PA1249	Alkaline Metalloproteinase AprA Precursor+	50	28	28	32	60.0%	11	11	16	14.0%
PA0572	Hypothetical Protein+	100	76	80	103	40.0%	11	11	12	13.0%
PA5339	Putative Endoribonuclease L-PSP	14	8	8	8	48.0%	11	13	26	46.0%
PA5240	Thioredoxin	12	20	20	20	47.0%	11	11	21	44.0%
PA2424	Peptide Synthase	480	32	32	32	7.2%	10	10	12	1.7%
PA2966	Acyl Carrier Protein	9	12	24	44	72.0%	10	11	21	67.0%
PA2451	Hypothetical Protein	58	99	100	188	61.0%	10	10	10	17.0%
PA3351	Putative Negative Regulator Of Flagellin Synthesis, FlgM+	11	17	20	28	63.0%	8	8	14	54.0%
PA1245	Conserved Hypothetical Protein	41	40	44	60	26.3%	6	6	7	6.8%
PA0041	Probable Hemagglutinin+	362	20	28	36	27.2%	6	6	6	3.7%
PA1093	Hypothetical Protein	13	19	20	48	54.0%	6	6	6	28.0%
PA4625	Hypothetical Protein+	203	12	12	12	8.0%	6	5	6	2.2%
PA1080	Flagellar Hook Protein FlgE+	48	32	32	36	60.0%	4	4	4	6.5%
PA5112	Esterase EstA+	69	4	4	6	3.5%	4	4	4	4.0%
PA1148	Exotoxin A Precursor+	69	40	44	56	52.0%	1	1	1	2.7%
PA2377	Putative ABC-Type Transport Protein, Periplasmic Component	44	28	36	40	65.0%	1	1	1	2.2%
PA2398	Ferripyoverdine Receptor+	91	36	40	52	34.0%	0	0	0	0.0%
PA4525	Type 4 Fimbrial Precursor PilA+		16	20	24	0.76	0	0	0	0.00%
PA0026	Phospholipase C, PlcB+	37	4	4	5	8.4%	0	0	0	0.0%
PA1156	NrdA, Catalytic Component Of Class Ia Ribonucleotide Reductase	107	0	0	0	0.0%	37	36	42	20.0%
PA0329	Conserved Hypothetical Protein	12	0	0	0	0.0%	16	18	28	65.0%
PA4366	Superoxide Dismutase+	21	0	0	0	0.0%	15	15	16	60.0%
AES_7139	Hypothetical Protein	17	0	0	0	0.0%	13	15	19	56.0%
PA0085	Conserved Hypothetical Protein+	17	0	0	0	0.0%	8	8	9	35.0%
PA2300	Chitinase ChiC+	53	0	0	0	0.0%	8	8	12	9.9%
PA2862	Lactonizing Lipase LipA Precursor+	31	0	0	0	0.0%	2	2	3	9.2%

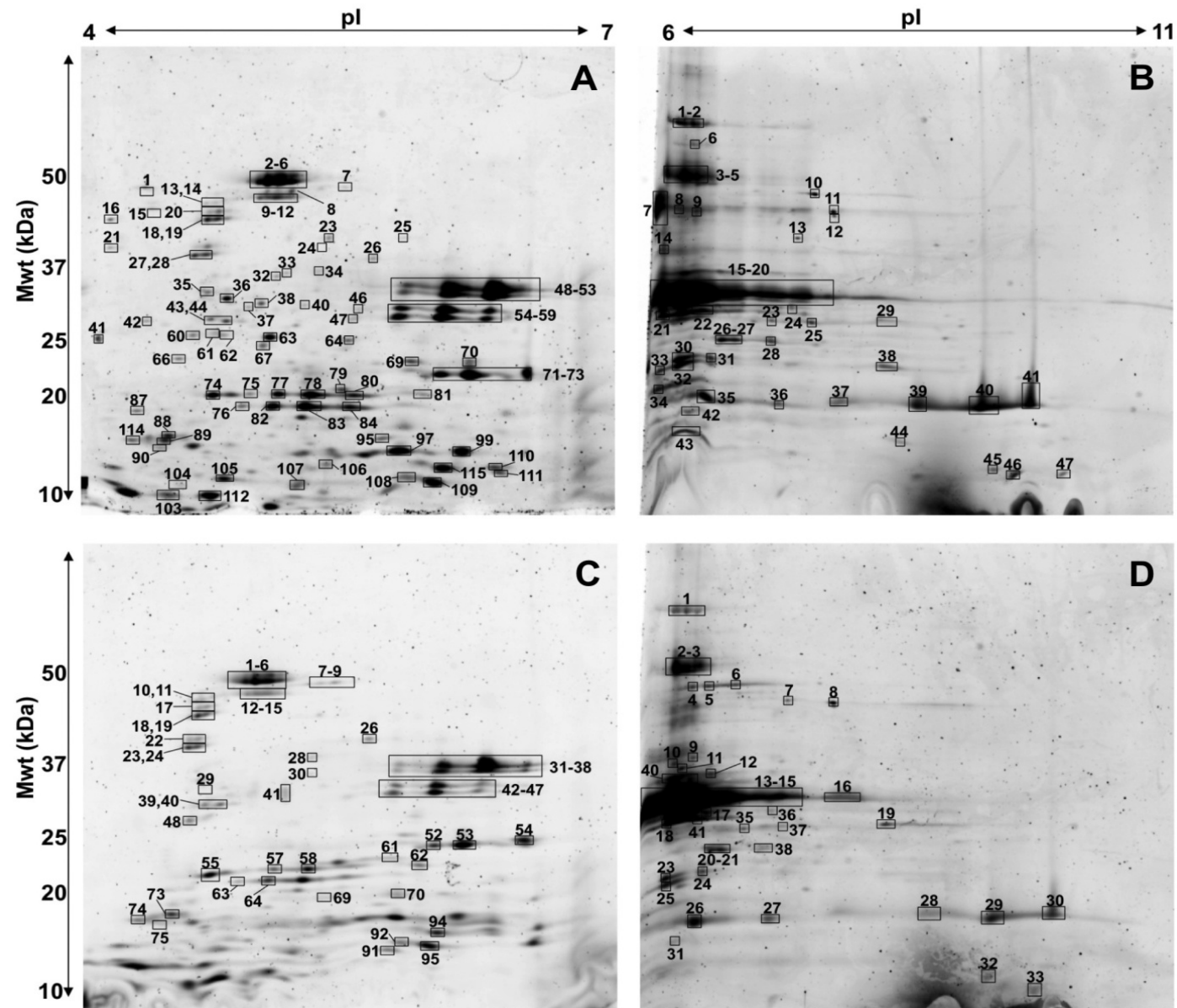
+ proteins denoted as non-cytoplasmic or extracellular from the PseudoCAP database
(www.pseudomonas.com)

Legends to Supplementary Figures

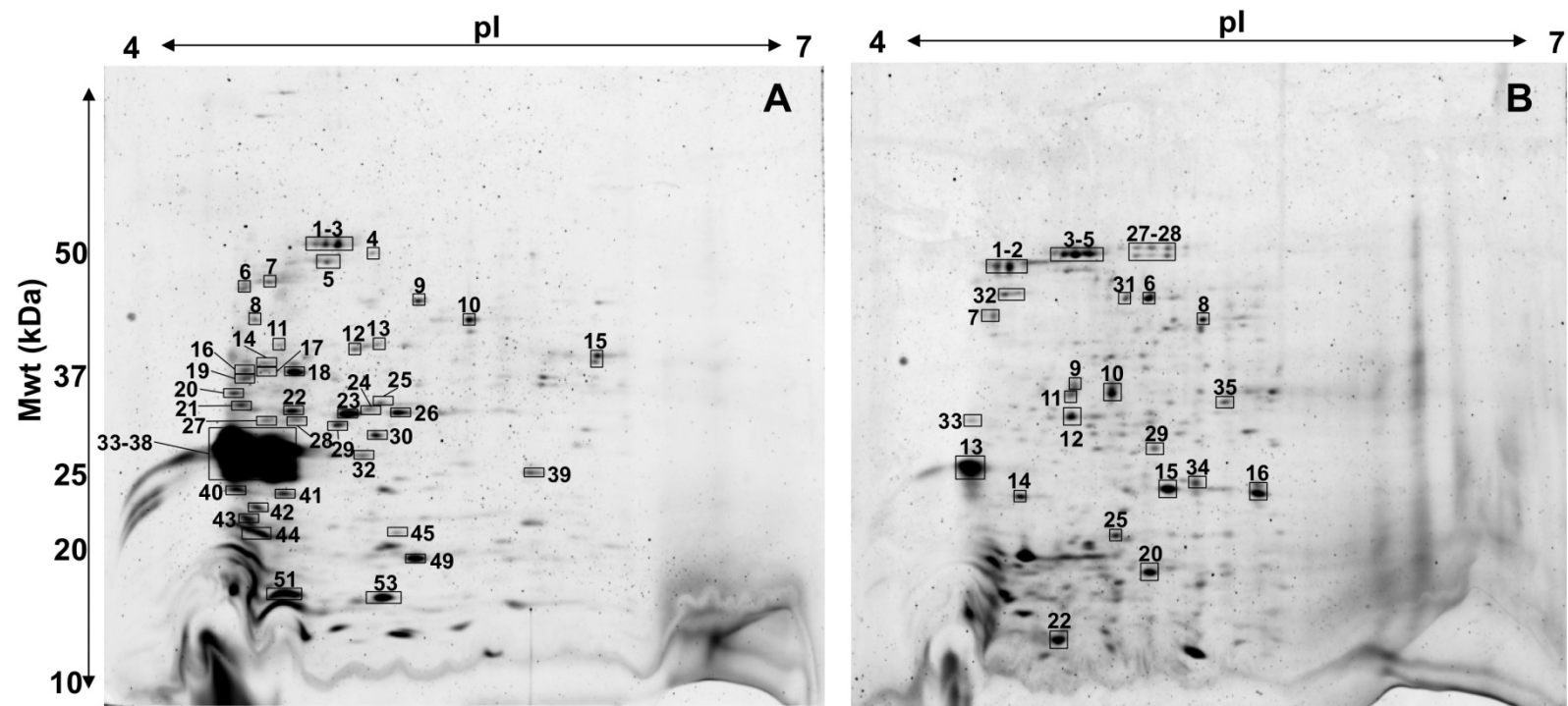
Supplementary Figure S1. 2-DE of culture supernatant proteins from *P. aeruginosa* PAO1 (A and B) and AES-1R (C and D) grown in LB medium and separated on pH 4-7 (A and C) and pH 6-11 (B and D) gels. Numbers correspond to spot identities shown in Suppl. Table S3.

Supplementary Figure S2. 2-DE of culture supernatant proteins from *P. aeruginosa* PAO1 (A and B) and AES-1R (C and D) grown in M9 medium and separated on pH 4-7 gels. Numbers correspond to spot identities shown in Suppl. Table S4.

Supplementary Figure S3. Genomic screening of *P. aeruginosa* CF isolates for *aes_7139*. Genomic DNA was extracted from overnight cultures and PCR conducted to amplify the full *aes_7139* gene (1, A, i and ii) and a 161 bp internal sequence (1, B, i and ii), and products were run on 1.5% (w/v) agarose gels. Strain AES-1R was used as a positive control, while strain PAO1 was used as a negative control for the presence of *aes_7139*. M, denotes GeneRuler 100 bp DNA ladder (Thermo Scientific, CA). As a positive control, we also screened the strains for *oprF* (2, i, ii and iii). PCR was conducted to amplify a 209 bp internal sequence (i, ii and iii)



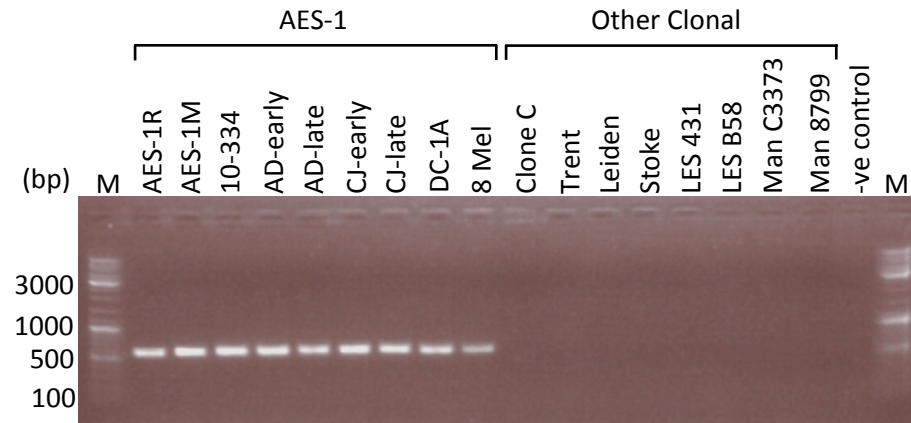
Supplementary Figure 1.



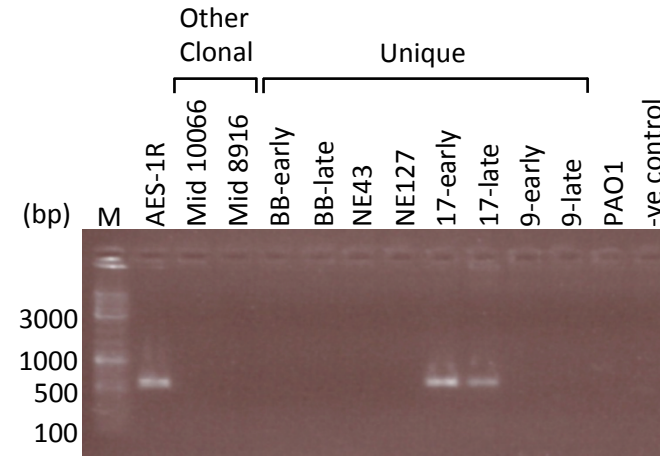
Supplementary Figure 2.

1

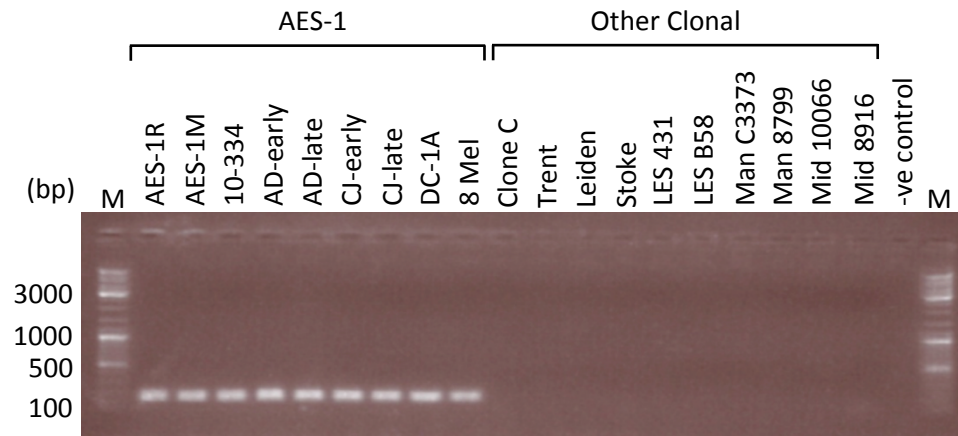
A i



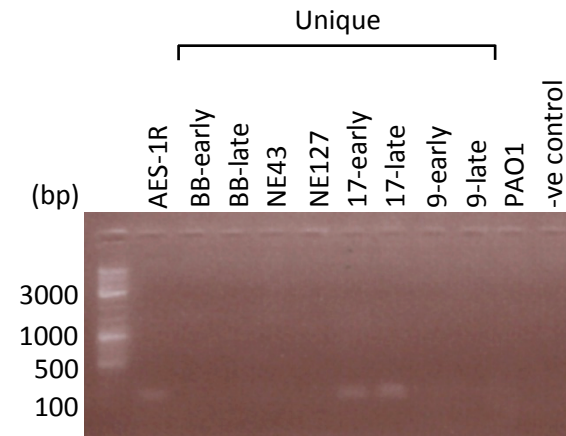
ii



B i

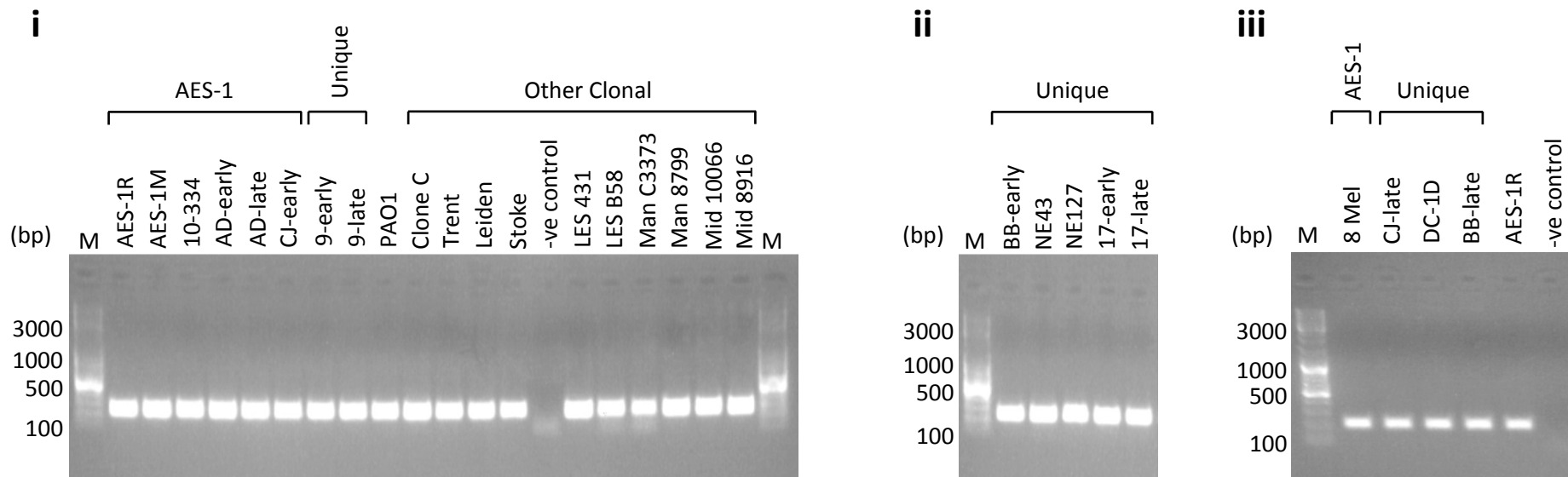


ii



Supplementary Figure S3.

2



Supplementary Figure S3.

Supplementary Data S1: Selected reaction monitoring (SRM) Data

PAO1 ID	AES-1R ID	Protein name	Peptide	AES-1R mean area	AES-1R sd	AES-1R CV	PAO1 Mean area	PAO1 sd	PAO1 CV	Fold change (AES-1R / PAO1)	t-test	Pages
PA0852	AES_1561	Chitin-Binding Protein CbpD Precursor	³⁶ VYGCFLEGPENPK ^{48*}	622176	11295	1.8%	77420	6814	8.8%	8.0	1.8E-11	2 and 3
PA3724	AES_5204	Elastase LasB	²⁸⁶ DWFGTSPLTHK ²⁹⁶	2682512	43587	1.6%	122353	6279	5.1%	21.9	1.1E-08	4 and 5
			²¹² YTYGSDYGPLIVNDR ²²⁶	2488305	53758	2.2%	503586	18553	3.7%	4.9	7.9E-09	6 to 8
PA4922	AES_6349	Azurin Precursor	¹¹³ DSVTFDVSK ^{121*}	439471	7270	1.7%	238194	13359	5.6%	1.8	6.8E-08	9 and 10
			⁹¹ DYLPDDSR ⁹⁹	193741	2131	1.1%	120500	2198	1.8%	1.6	1.7E-11	11 and 12
PA4495	AES_5942	Conserved Hypothetical Protein	¹¹⁴ LESSDFAALGQLSADLLGK ¹³²	142405	5639	4.0%	37062	2174	5.9%	3.8	1.4E-07	13 and 14
PA4625	AES_6066	Cyclic Diguanylate-Regulated Two-Partner Secretion System (TPS) Partner A CdrA	VYGDADPSLTQVSGLK*	11556	512	4.4%	214936	14160	6.6%	-18.6	5.5E-06	15 and 16
PA1777	AES_2937	Major Porin and Structural Outer Membrane Porin OprF Precursor	³⁰² DVLVNEYGVEGGR ³¹⁴	228648	7493	3.3%	160291	8142	5.1%	1.4	7.8E-07	17 and 18
PA1342	AES_2411	Probable Binding Protein Component of ABC Transporter	²⁵⁰ AITDTYASGEVSK ²⁶²	59276	2660	4.5%	10509	894	8.5%	5.6	2.8E-07	19 and 20
PA2939	AES_4384	Probable Aminopeptidase	⁴⁸ SPLLSTPLGLPR ⁶⁰	1452098	38036	2.6%	2037753	197726	9.7%	-1.4	2.2E-03	21 and 22
			¹²⁴ GPGSLSATVPQPVTYWEK ¹⁴²	239677	9670	4.0%	374540	34118	9.1%	-1.6	5.3E-04	23 and 24
PA0423	AES_0809	PasP Protease	¹⁰³ NPTATFESTEVK ¹¹⁴	81323	5898	7.3%	36385	2612	7.2%	2.2	9.1E-06	25 and 26
PA4175	AES_1026	PvdS-Regulated Endoproteinase, Lysyl Class	⁴²⁶ GGLYGGPSYCGAPTSQR ^{442*}	38044	1383	3.6%	39956	3082	7.7%	-1.1	2.6E-01	27 and 28
			⁴⁴³ NDYFSDFGVYSQISR ⁴⁵⁸	156127	3921	2.5%	143595	11031	7.7%	1.1	6.2E-02	29 and 30

Proteins and peptides used for SRM experiments. The resulting mean, standard deviation (Sd) and coefficient of variation (CV) of peptides are shown. Peptide labelled with * denotes peptide not seen with the initial 2DLC-MS/MS experiment and selected using *in silico* analysis with Skyline. Lower panels of following figures show replicate analysis of AES-1R (left, run 1-5) and PAO1 (right, run 1-5).

AES_1561 - Chitin-Binding Protein CbpD Precursor - PA0852

Peptide Sequence: VYGCFLEGPENPK

Parent Mass / Charge: 755.36 / +2

Transition list

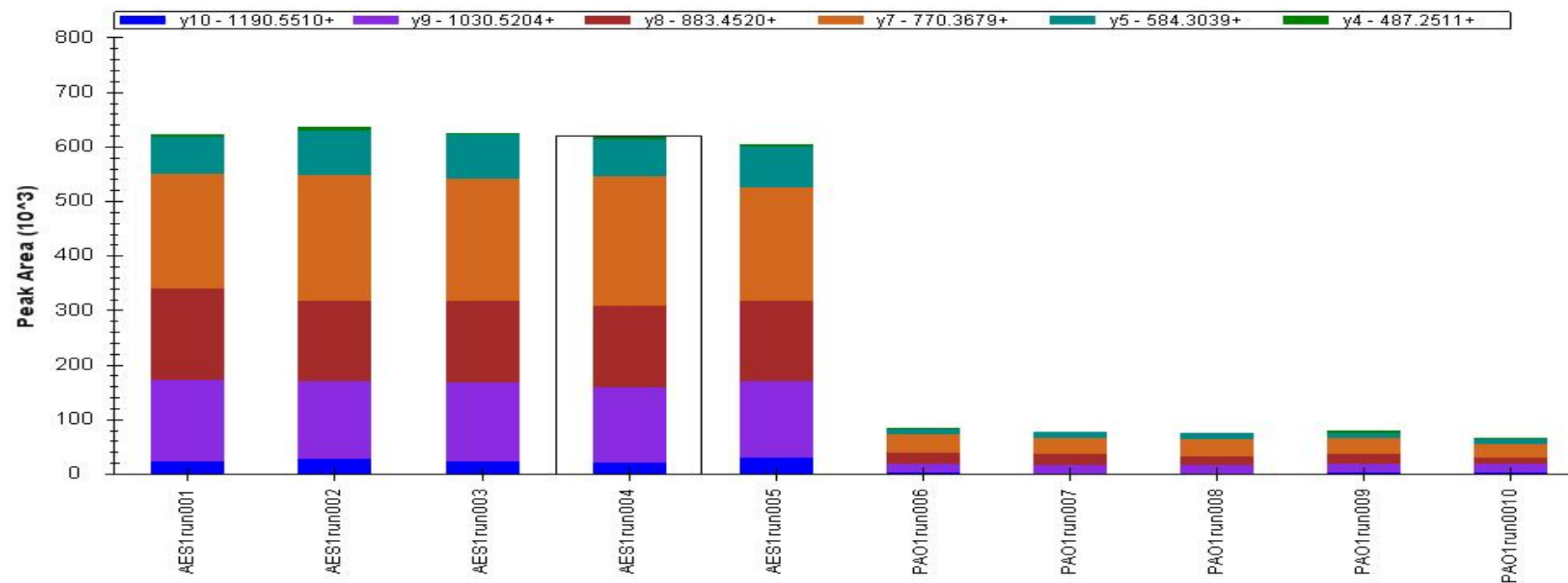
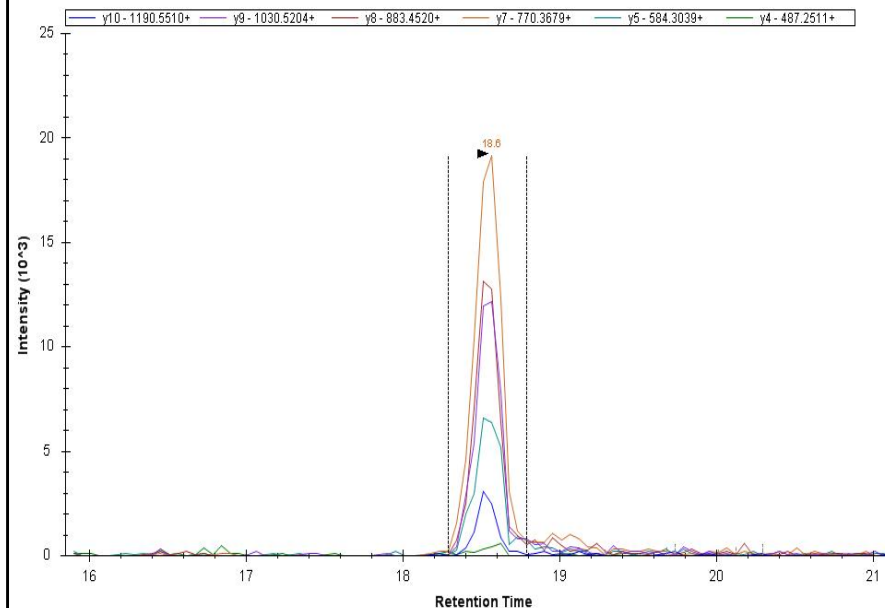
y10	1190.551035
y9	1030.520387
y8	883.451973
y7	770.367909
y5	584.303852
y4	487.251088

AES-1R mean area: 622176 PAO1 mean area: 77420.2

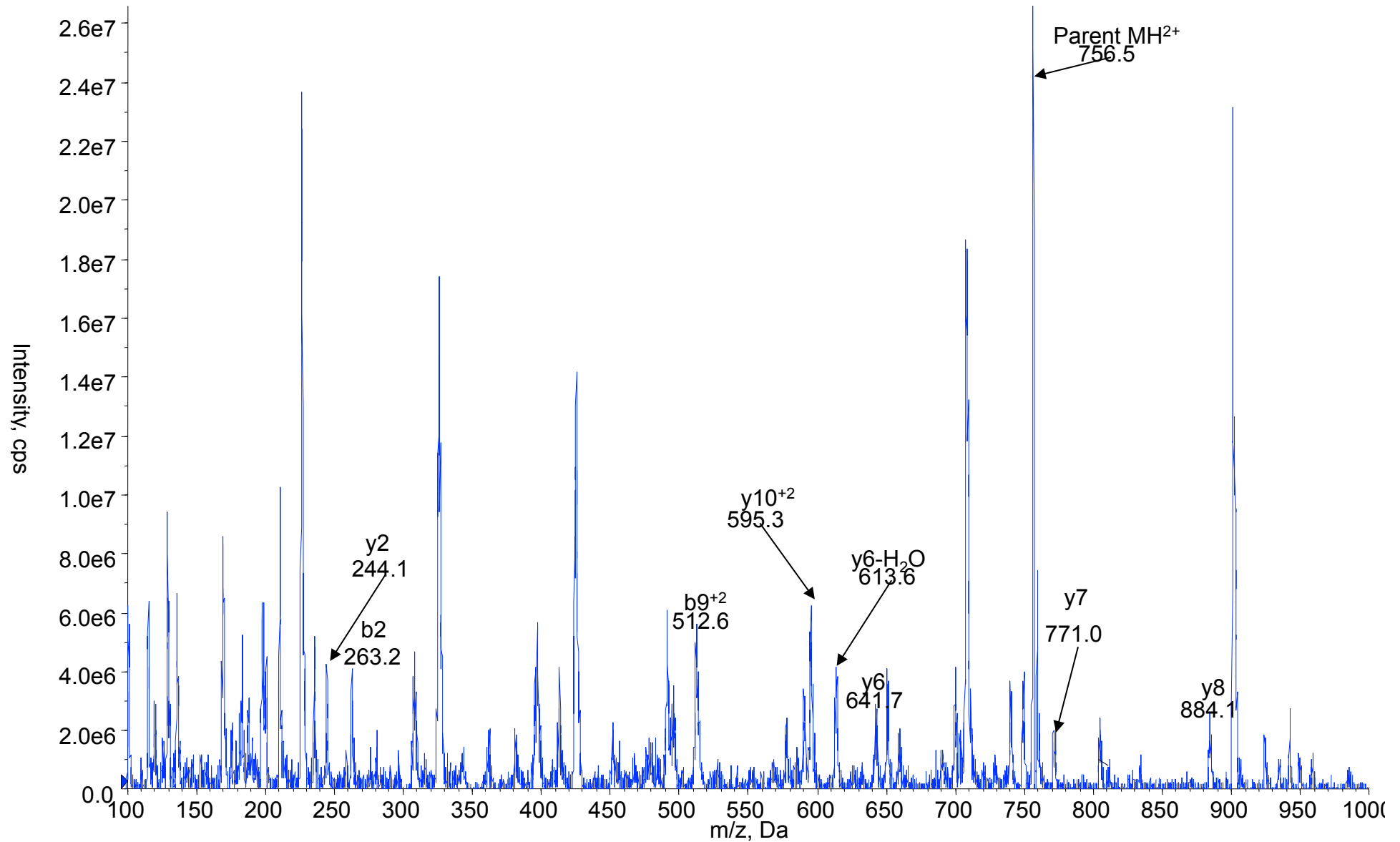
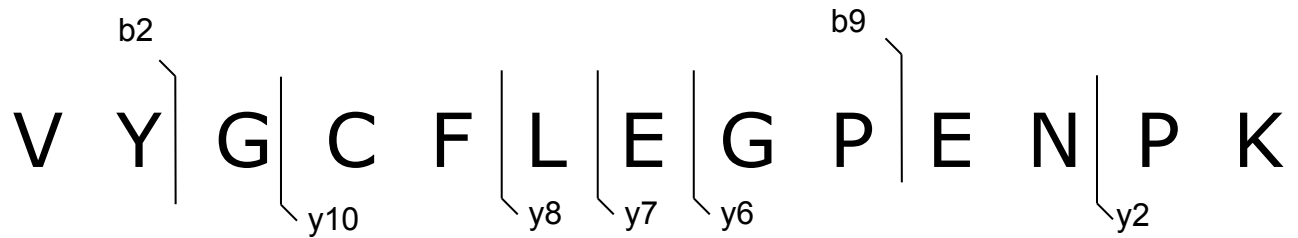
AES-1R Stdev: 11295 PAO1 Stdev: 6813.8

AES-1R CV: 1.82% PAO1 CV: 8.80%

T-TEST: $p = 1.77 \times 10^{-11}$ Ratio of AES-1R to PAO1: 8.04



Protein: CbpD Precursor - PA0852	
Precursor charge: +2	
Precursor m/z: 755.36	
Mascot Ion score: NA	



AES_5204 - Elastase LasB - PA3724

Peptide Sequence: DWFGTSPLTHK

Parent Mass / Charge: 644.82 / +2

Transition list

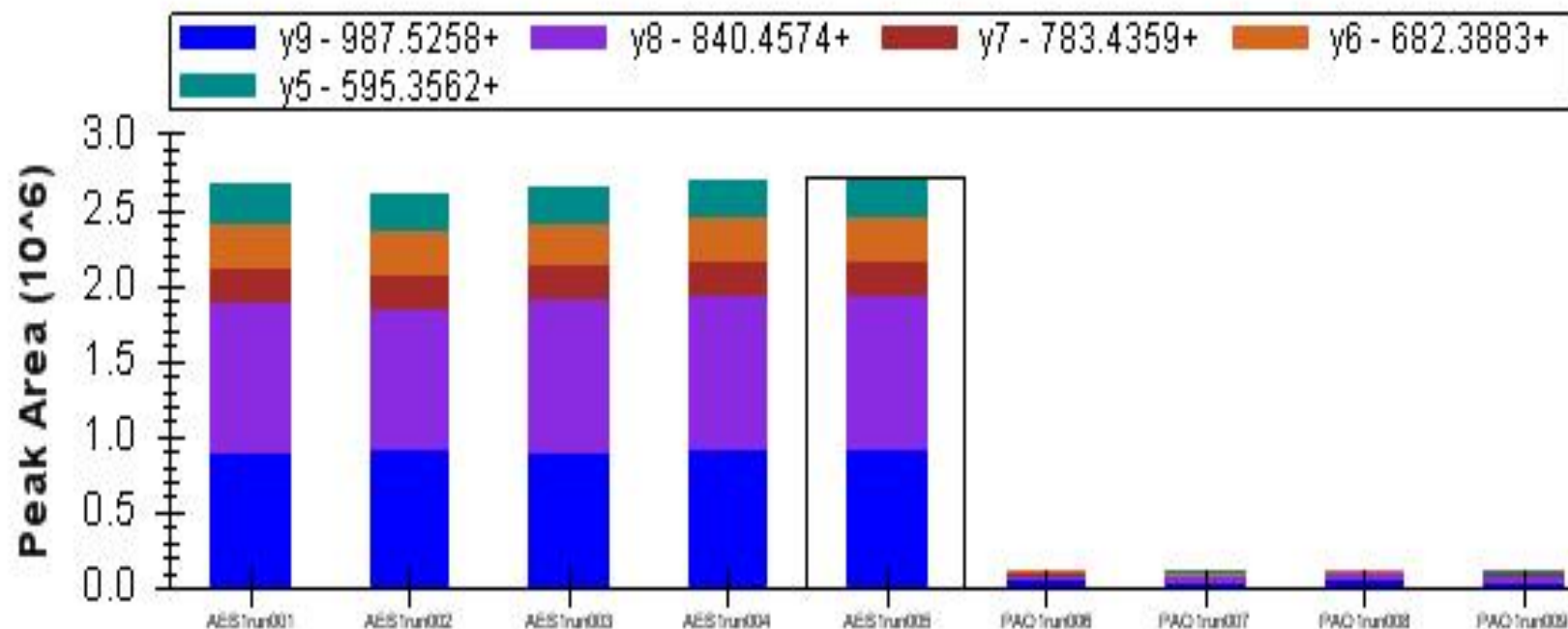
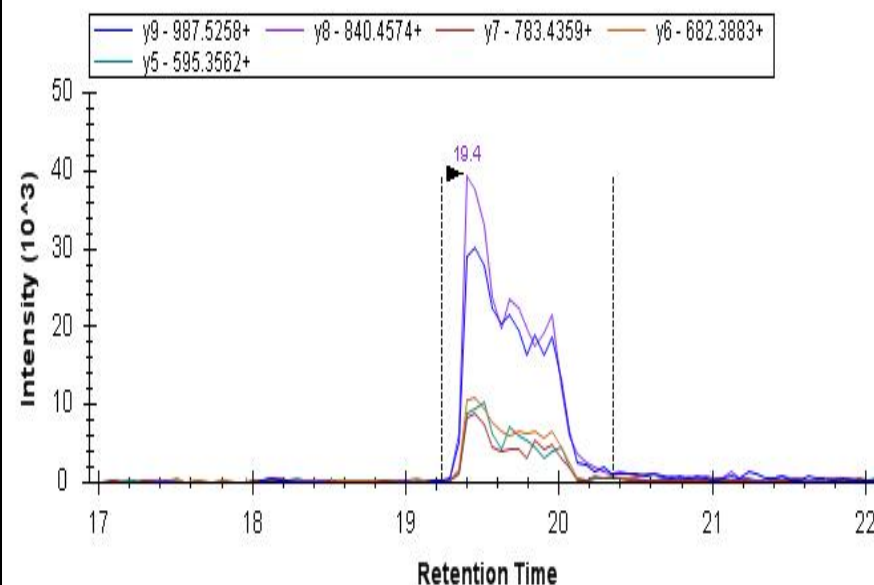
y9	987.525807
y8	840.457393
y7	783.435929
y6	682.38825
y5	595.356222

AES-1R mean area: 2682511.8 PAO1 mean area: 122352.6

AES-1R Stdev: 43587 PAO1 Stdev: 6279.2

AES-1R CV: 1.6% PAO1 CV: 5.1%

T-TEST: $p = 1.12 \times 10^{-08}$ Ratio of AES-1R to PAO1: 21.9



Protein: Elastase LasB - PA3724	
Precursor charge: +2	
Precursor m/z: 644.82	
Mascot Ion score: 44	

a2/b2

b3

b4

b6

b8

D

W

F

G

T

S

P

L

T

H

K

y9

y8

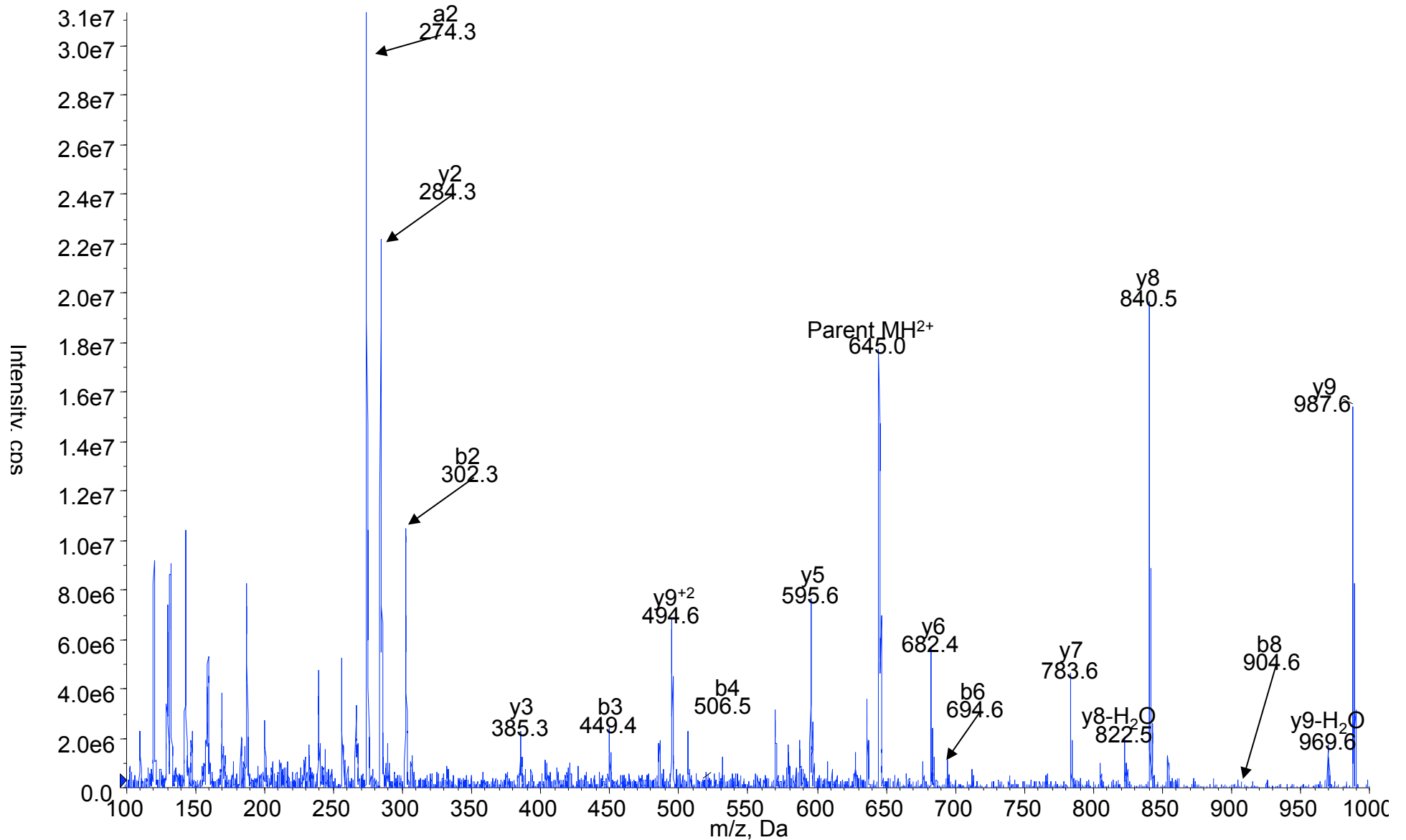
y7

y6

y5

y3

y2



AES_5204 - Elastase LasB - PA3724

Peptide Sequence: YTYGSDYGPLIVNDR

Parent Mass / Charge: 866.91 / +2

Transition list

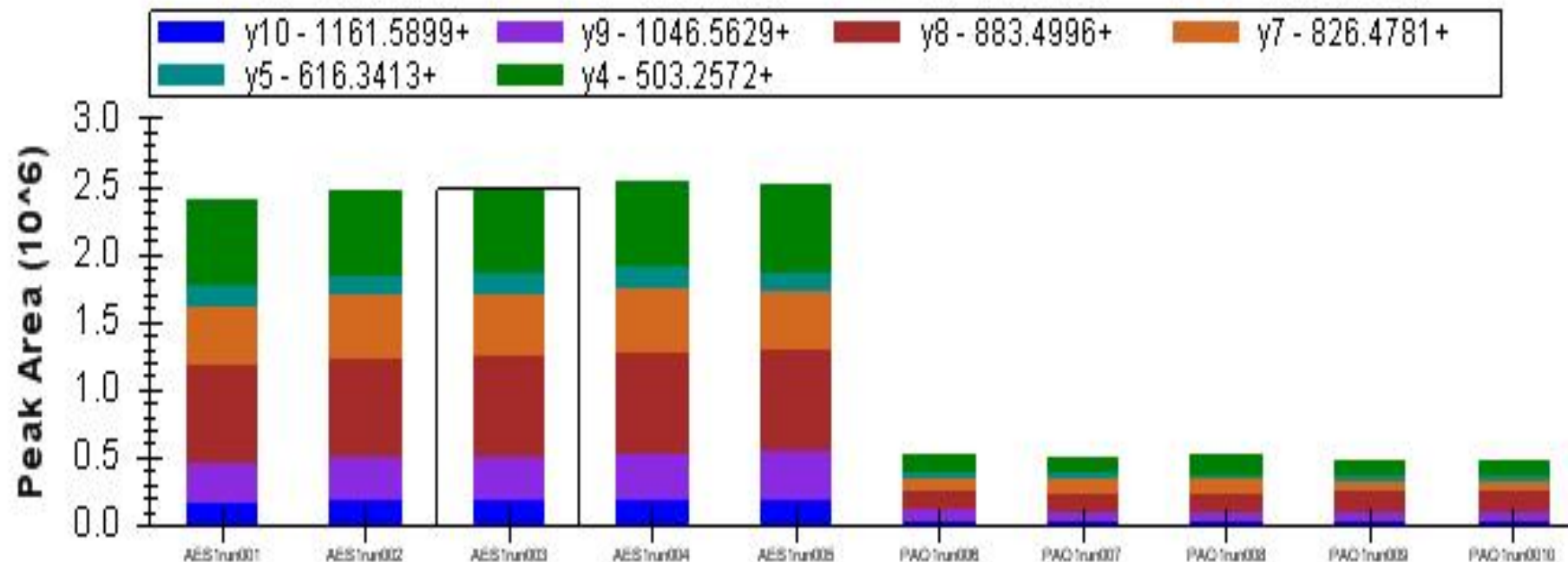
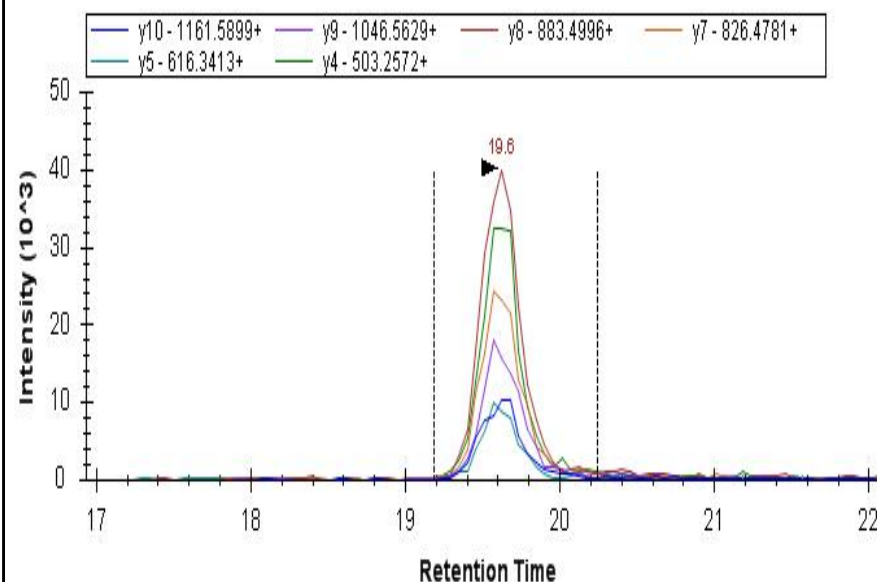
y10	1161.58986
y9	1046.56292
y8	883.499592
y7	826.478128
y5	616.3413
y4	503.257236

AES-1R mean area: 2488305.2 PAO1 mean area: 503586.2

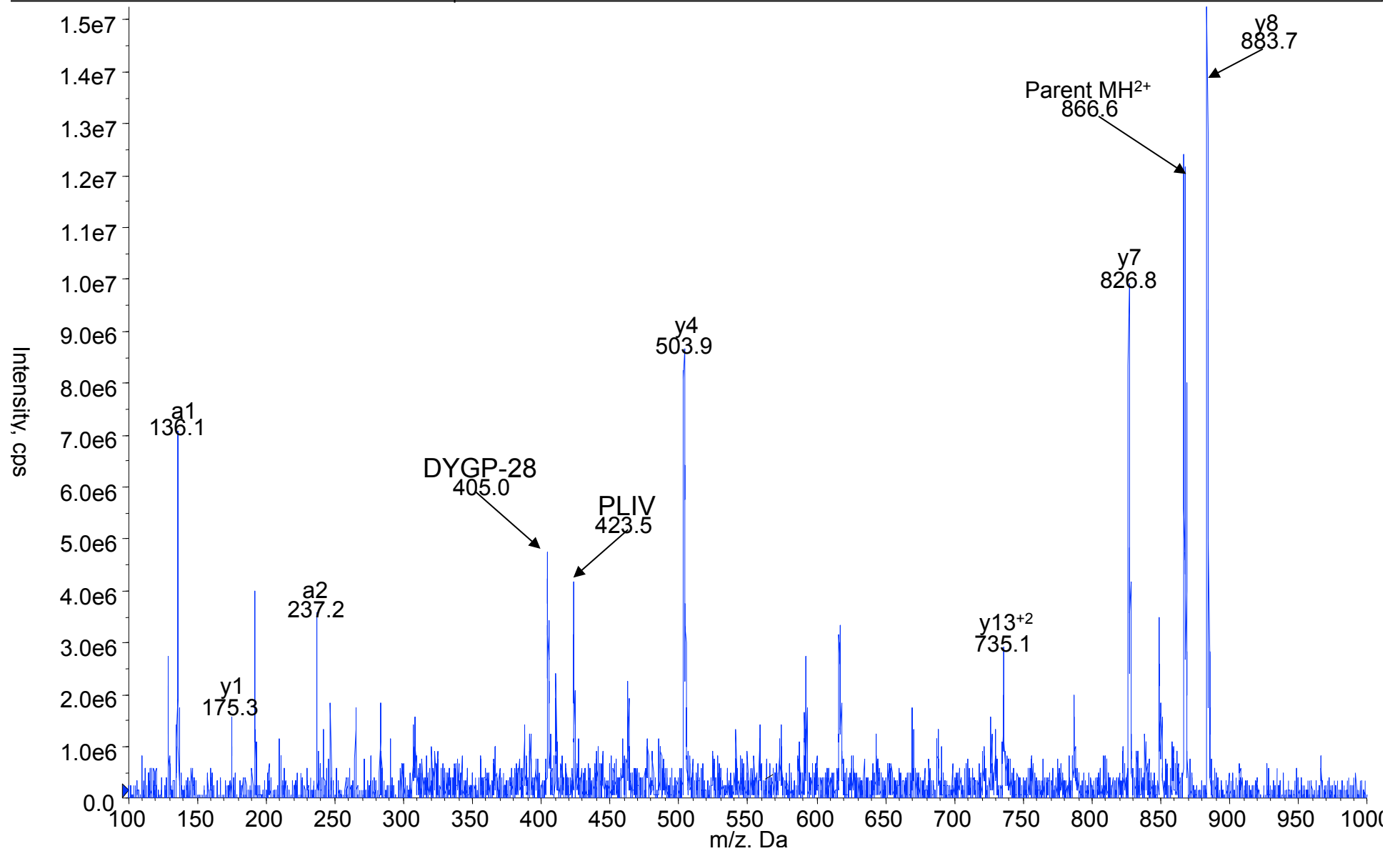
AES-1R Stdev: 53758 PAO1 Stdev: 18553.0

AES-1R CV: 2.2% PAO1 CV: 3.7%

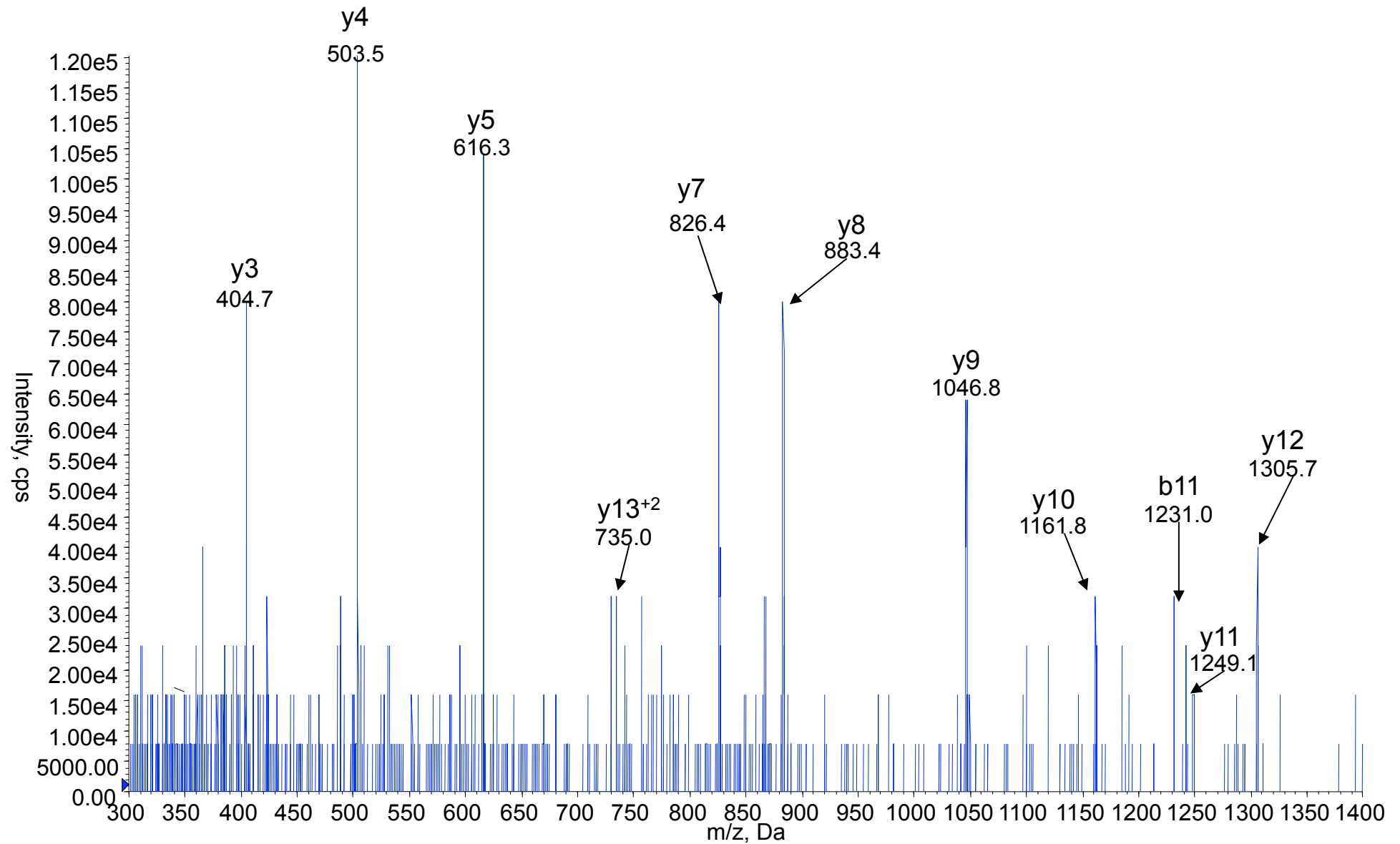
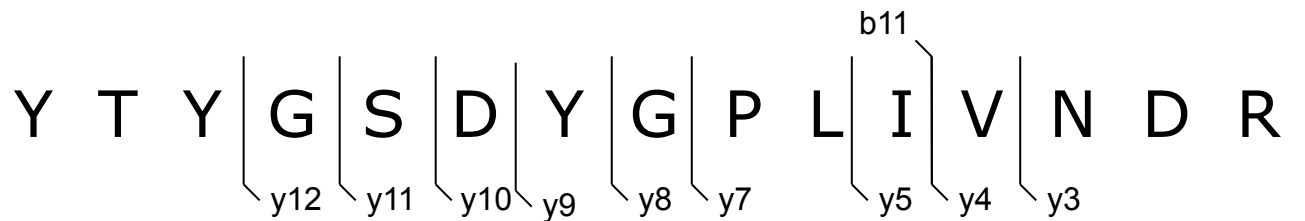
T-TEST: $p = 7.92 \times 10^{-09}$ Ratio of AES-1R to PAO1: 4.9



Protein: Elastase LasB - PA3724	a1	a2													
Precursor charge: +2	Y	T	Y	G	S	D	Y	G	P	L	I	V	N	D	R
Precursor m/z: 866.91															
Mascot Ion score: 17															



Protein: Elastase LasB - PA3724	
Precursor charge: +2	
Precursor m/z: 866.91	
Mascot Ion score: 50	



AES_6349 - Azurin Precursor - PA4922

Peptide Sequence: DSVTFDVSK

Parent Mass / Charge: 499.24 / +2

Transition list

y7	795.424696
y6	696.356282
y5	595.308603
y4	448.240189

AES-1R mean area: 439470.8 PAO1 mean area: 238193.6

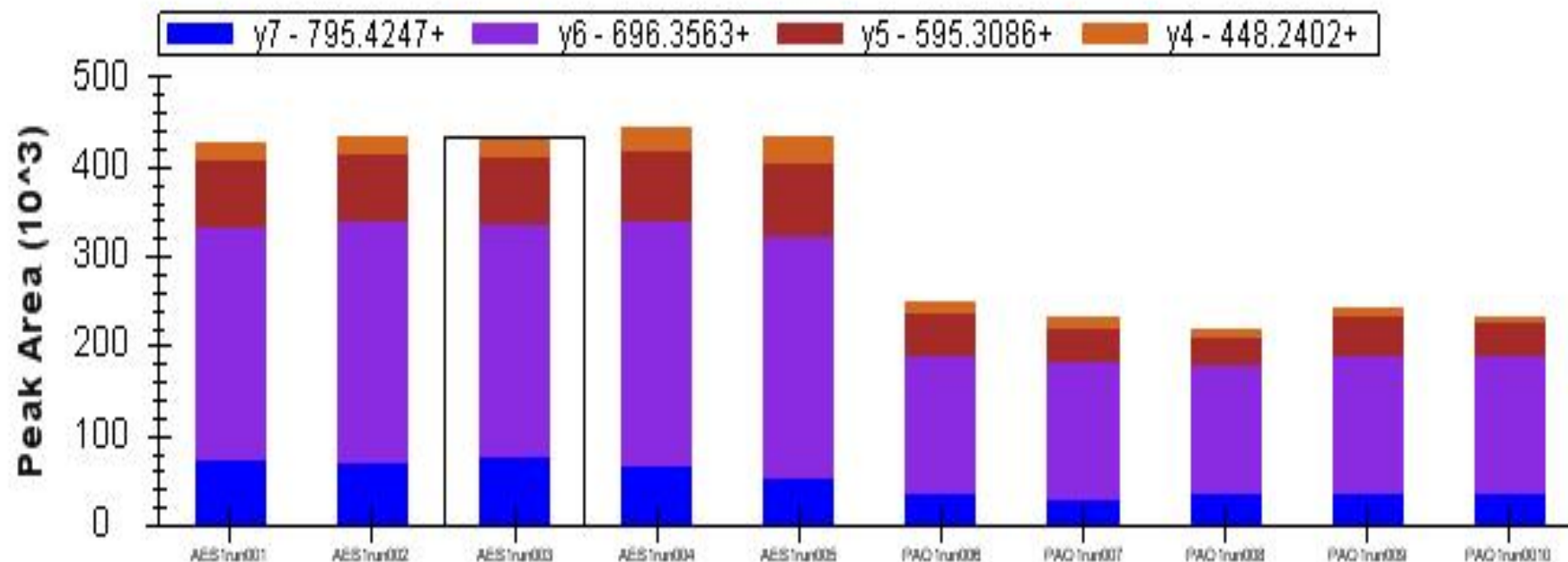
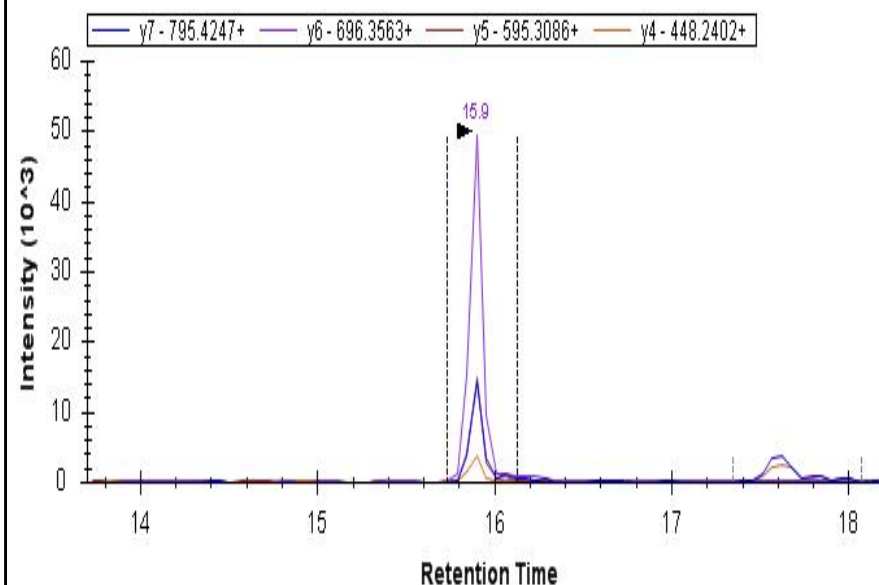
AES-1R Stdev: 7270

PAO1 Stdev: 13358.9

AES-1R CV: 1.7%

PAO1 CV: 5.6%

T-TEST: $p = 6.83 \times 10^{-08}$ Ratio of AES-1R to PAO1: 1.8

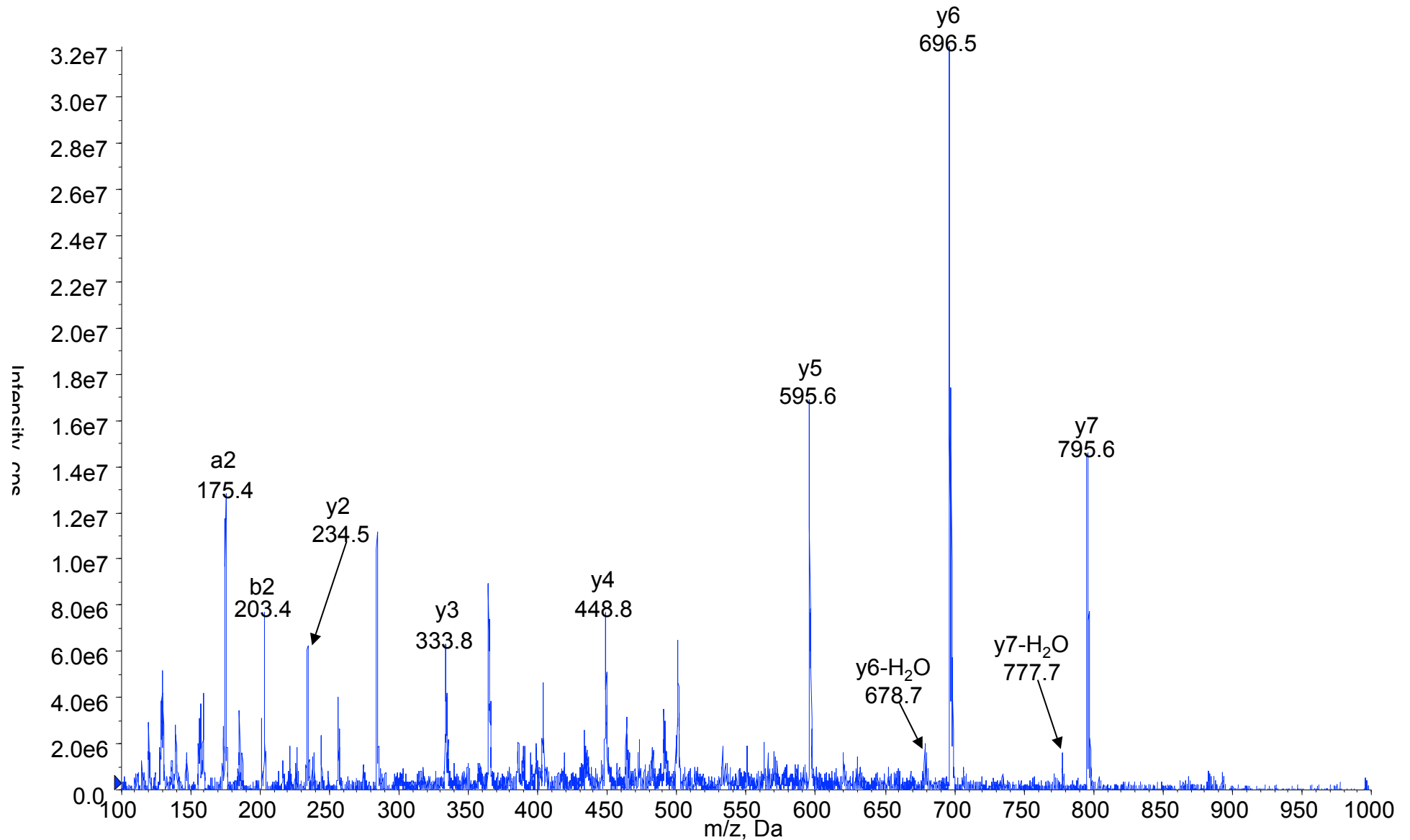


Protein: Azurin Precursor - PA4922	
Precursor charge: +2	
Precursor m/z: 499.24	
Mascot Ion score: 33	

a2/b2

D
S
V
T
F
D
V
S
K

y7
y6
y5
y4
y3
y2



AES_6349 - Azurin Precursor - PA4922

Peptide Sequence: DYLPKDDSR
Parent Mass / Charge: 554.77 / +2
Transition list

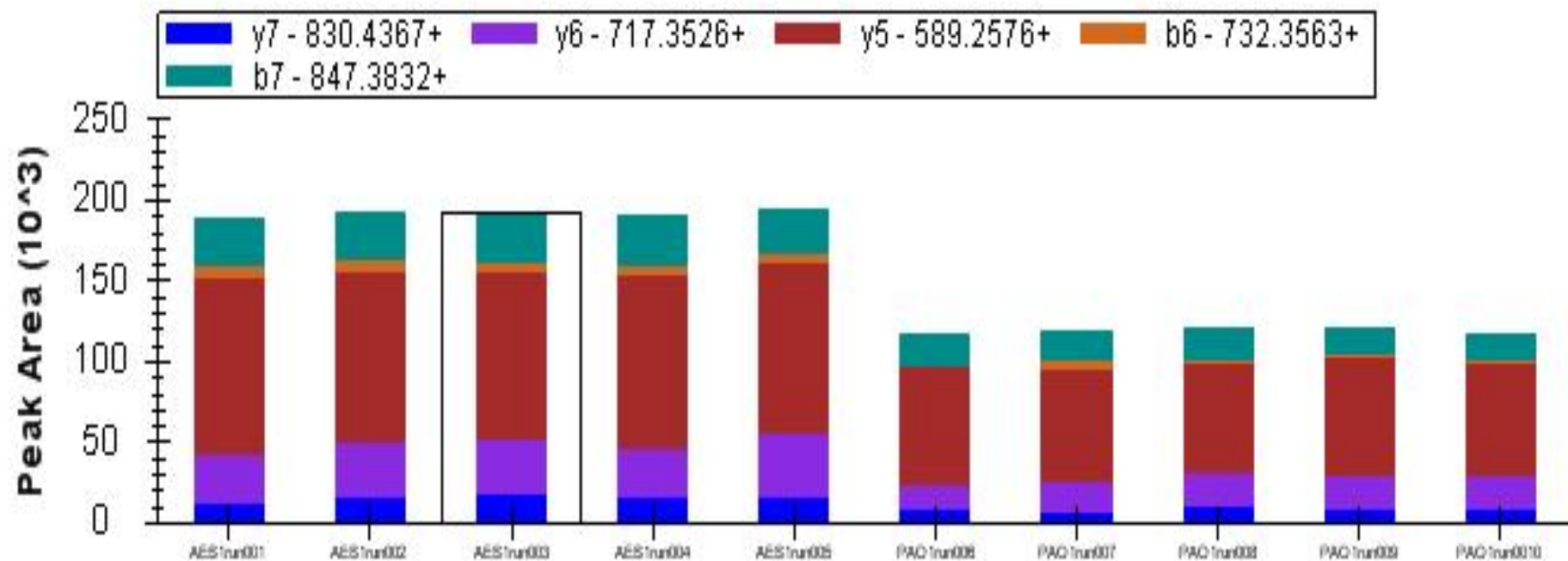
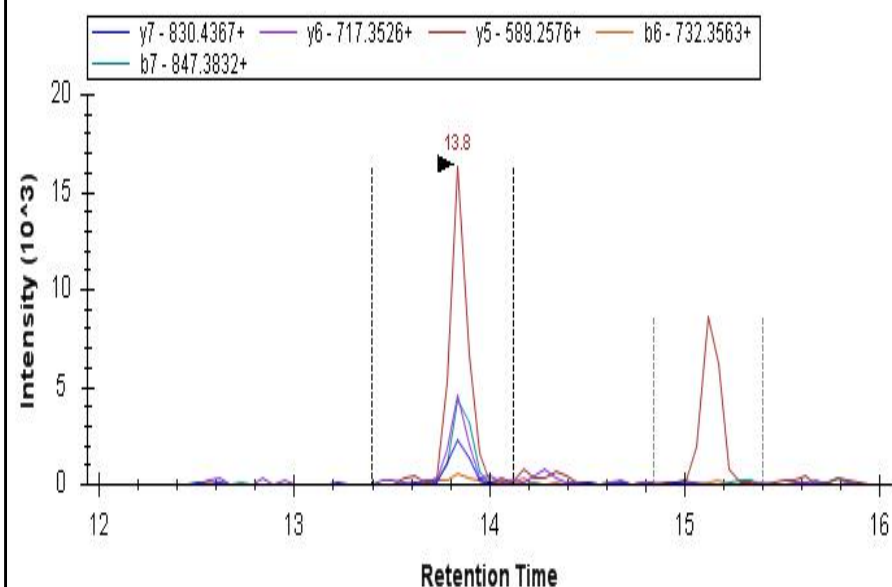
y7	830.43657
y6	717.352593
y5	589.25763
b6	732.356282
b7	847.383225

AES-1R mean area: 193740.8 PAO1 mean area: 120500.4

AES-1R Stdev: 2131 PAO1 Stdev: 2197.7

AES-1R CV: 1.1% PAO1 CV: 1.8%

T-TEST: $p = 1.68 \times 10^{-11}$ Ratio of AES-1R to PAO1: 1.6



Protein: Azurin Precursor - PA4922	
Precursor charge: +2	
Precursor m/z: 554.77	
Mascot Ion score: 29	

a2/b2

b5

b7

D

Y

L

K

P

D

D

S

R

y7

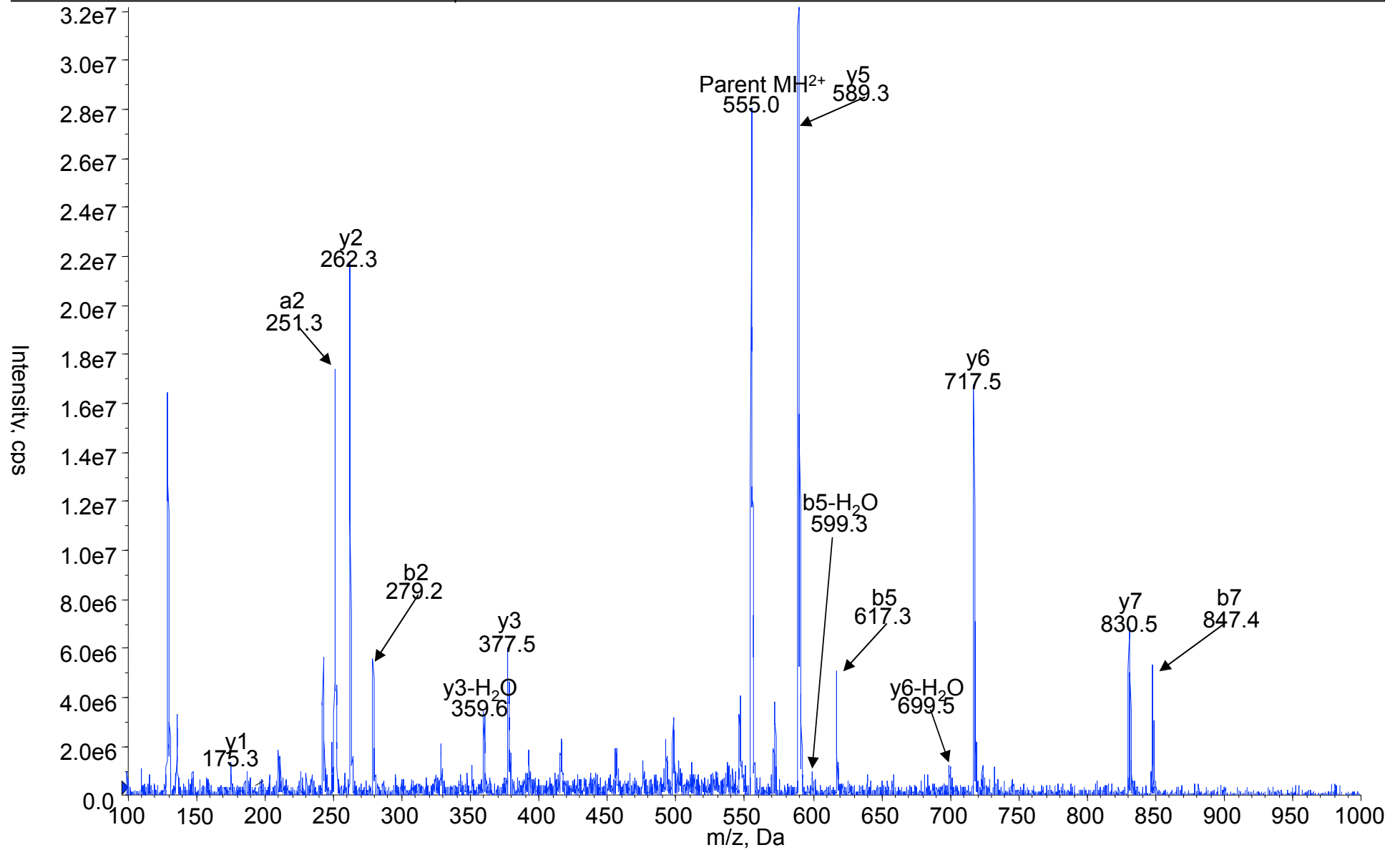
y6

y5

y3

y2

y1



AES_5942 - Conserved Hypothetical Protein - PA4495

Peptide Sequence: LESSDFAALGQLSADLLGK

Parent Mass / Charge: 968.01 / +2

Transition list

y13	1256.72088
y12	1185.68376
y11	1114.64665
y10	1001.56259
y9	944.541122
y8	816.482545

AES-1R mean area: 142405

PAO1 mean area: 37062

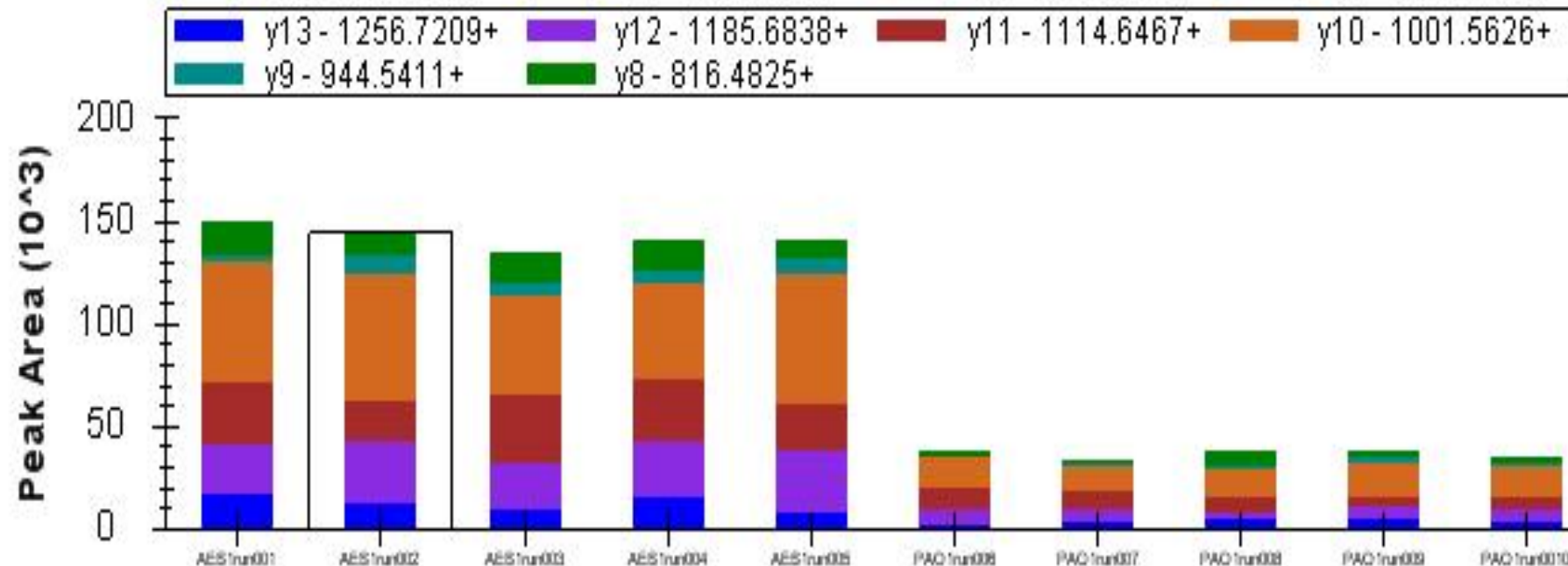
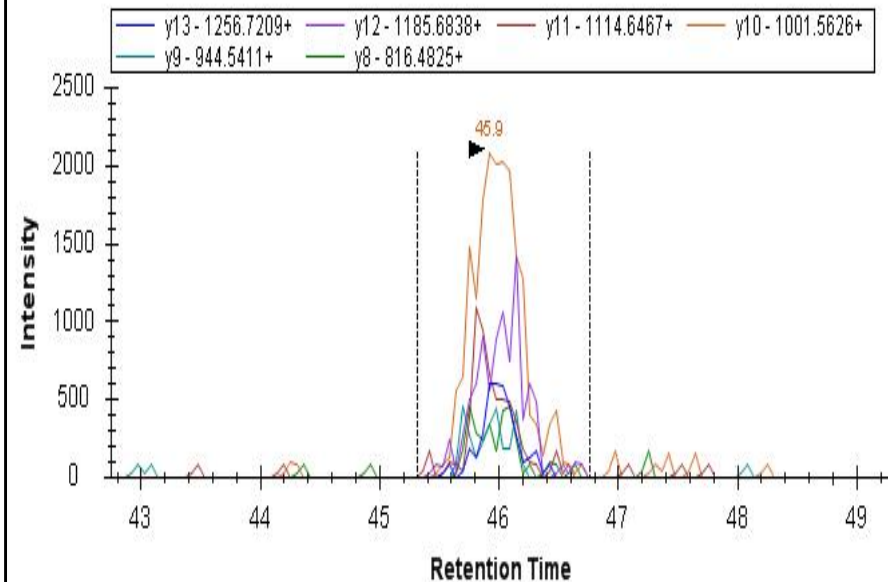
AES-1R Stdev: 5639

PAO1 Stdev: 2174

AES-1R CV: 4.0%

PAO1 CV: 5.9%

T-TEST: $p = 1.41 \times 10^{-7}$ Ratio of AES-1R to PAO1: 3.8



AES_6066 - Cyclic Diguanylate-Regulated Two-Partner Secretion System (TPS) Partner A CdrA - PA4625

Peptide Sequence: VYGDADPSLT YQVSGLK

Parent Mass / Charge: 906.95 / +2

Transition list

b6	621.251482
y11	1192.65722
y10	1095.60445
y9	1008.57242
y8	895.488358
y7	794.44068

AES-1R mean area: 11556

PAO1 mean area: 214936.2

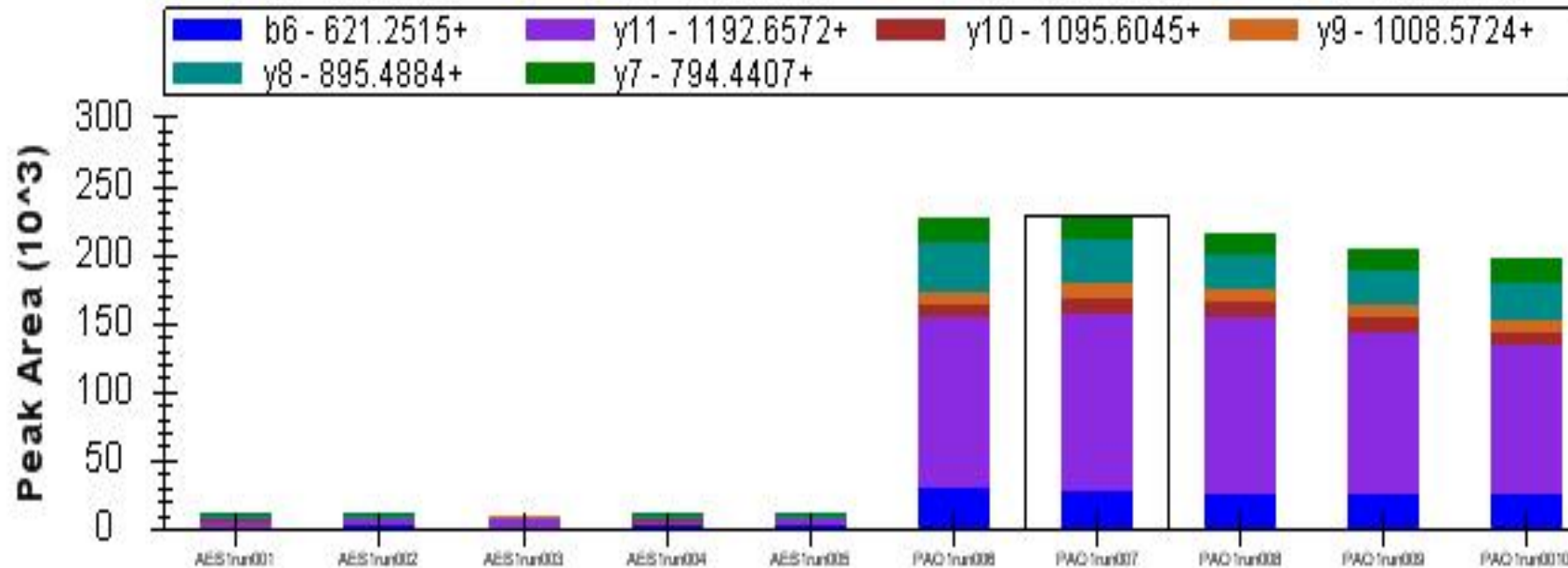
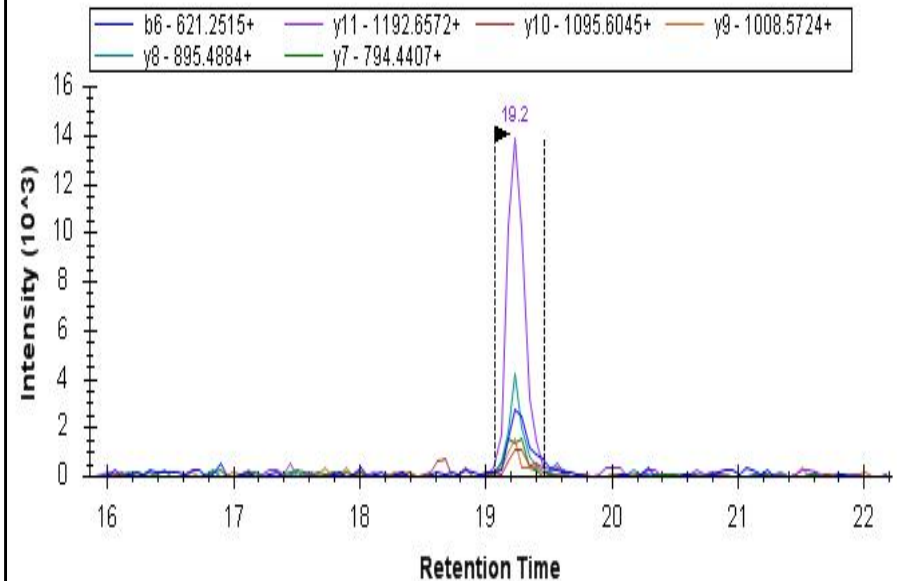
AES-1R Stdev: 512

PAO1 Stdev: 14160

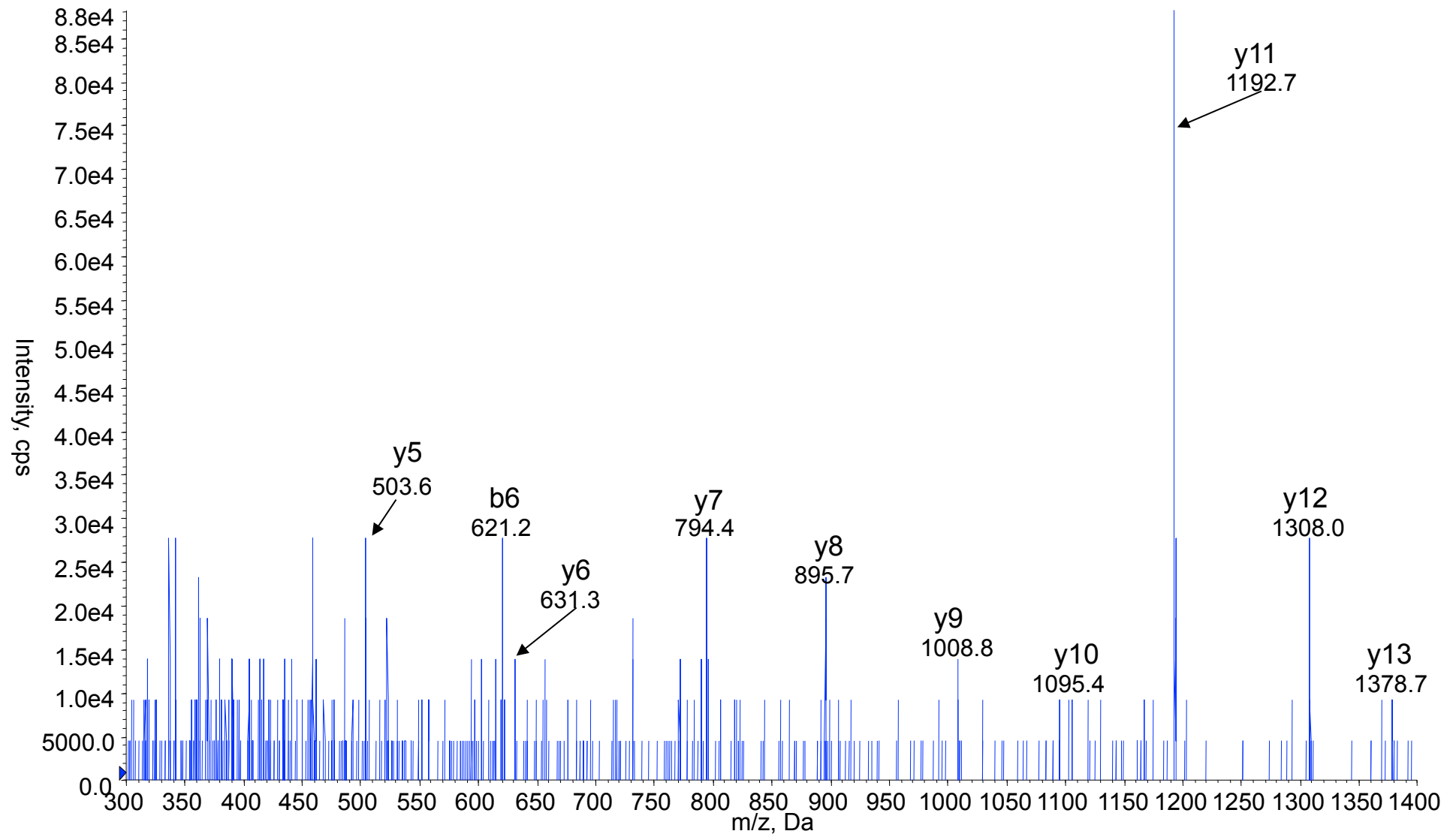
AES-1R CV: 4.4%

PAO1 CV: 6.6%

T-TEST: $p = 5.479 \times 10^{-6}$ Ratio of AES-1R to PAO1: 0.05



Protein: CdrA - PA4625	<pre> b6 V Y G D A [D] P S L T Y Q V S G L K y12 y11 y10 y9 y8 y7 y6 y5 </pre>
Precursor charge: +2	
Precursor m/z: 906.95	
Mascot Ion score: 55	



AES_2937 - Outer Membrane Porin OprF Precursor - PA1777

Peptide Sequence: DVLVNEYGVEGGR

Parent Mass / Charge: 703.85 / +2

Transition list

y11	1192.59568
y10	1079.51161
y9	980.443199
y8	866.400272
y7	737.357679
y6	574.29435

AES-1R mean area: 228648

PAO1 mean area: 160291.2

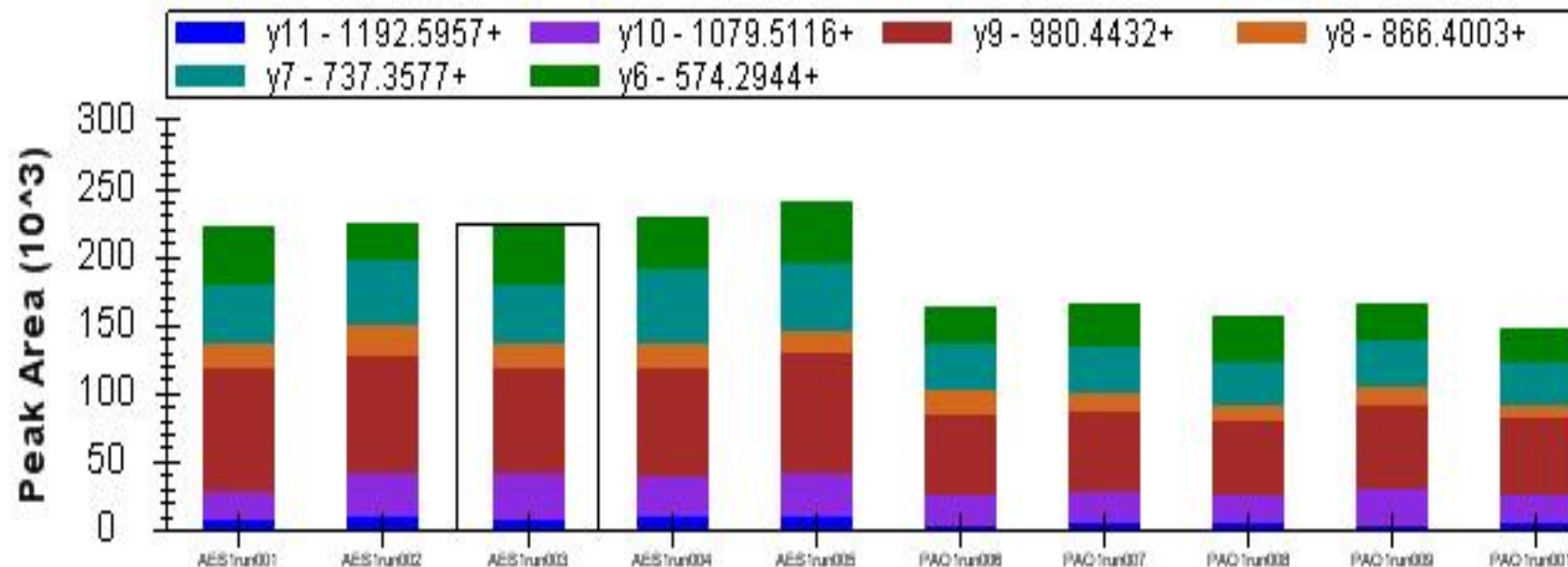
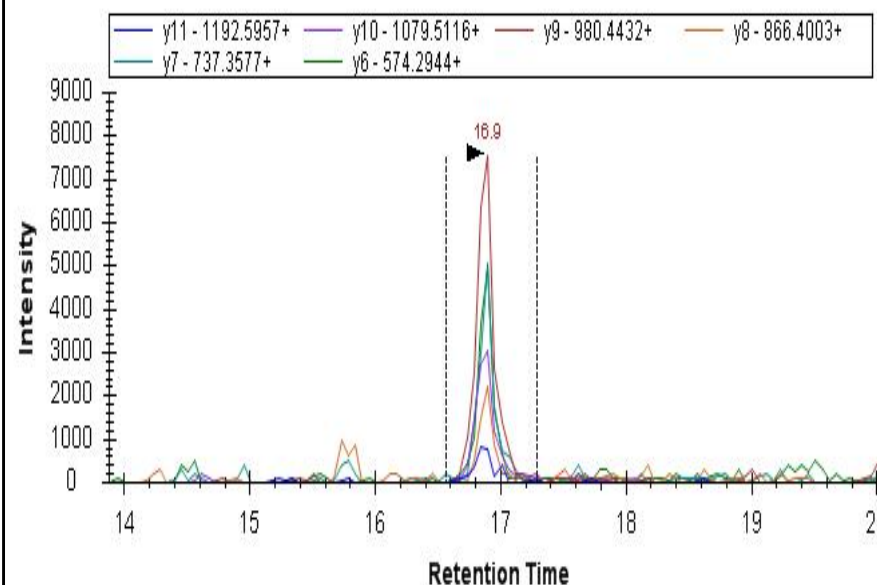
AES-1R Stdev: 7493

PAO1 Stdev: 8142

AES-1R CV: 3.3%

PAO1 CV: 5.1%

T-TEST: $p = 7.77 \times 10^{-7}$ Ratio of AES-1R to PAO1: 1.4

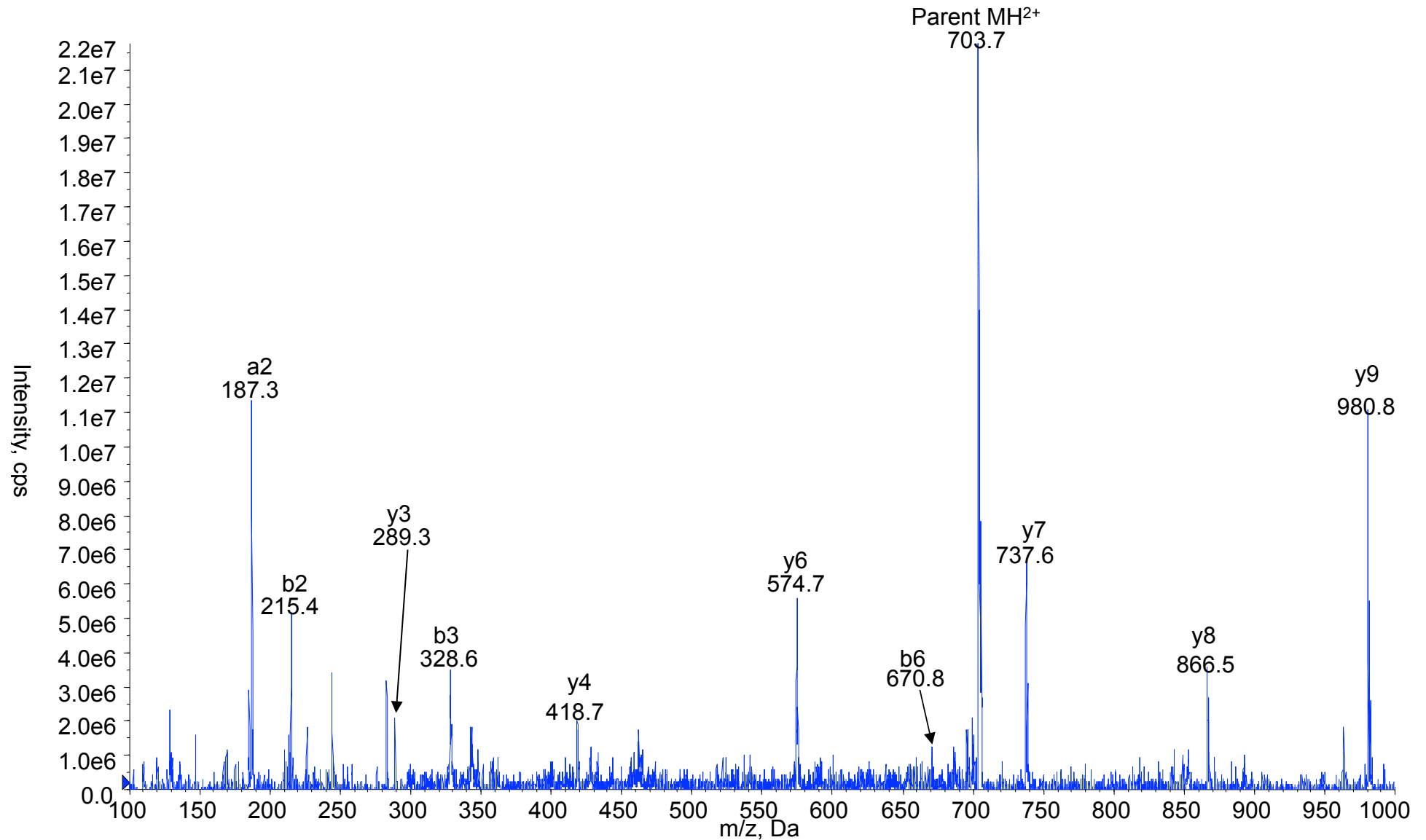


Protein: OprF Precursor - PA1777	
Precursor charge: +2	
Precursor m/z: 703.85	
Mascot Ion score: 26	

a2/b2
b3

D V L V N E Y G V E G G R

y9
y8
y7
y6
y4
y3



AES_2411 - Binding Protein Component of ABC Transporter - PA1342

Peptide Sequence: AITDTYASGEVNK

Parent Mass / Charge: 684.84 / +2

Transition list

y10	1083.49529
y9	968.468351
y8	867.420673
y7	704.357344
y6	633.32023

AES-1R mean area: 59276

PAO1 mean area: 10508.8

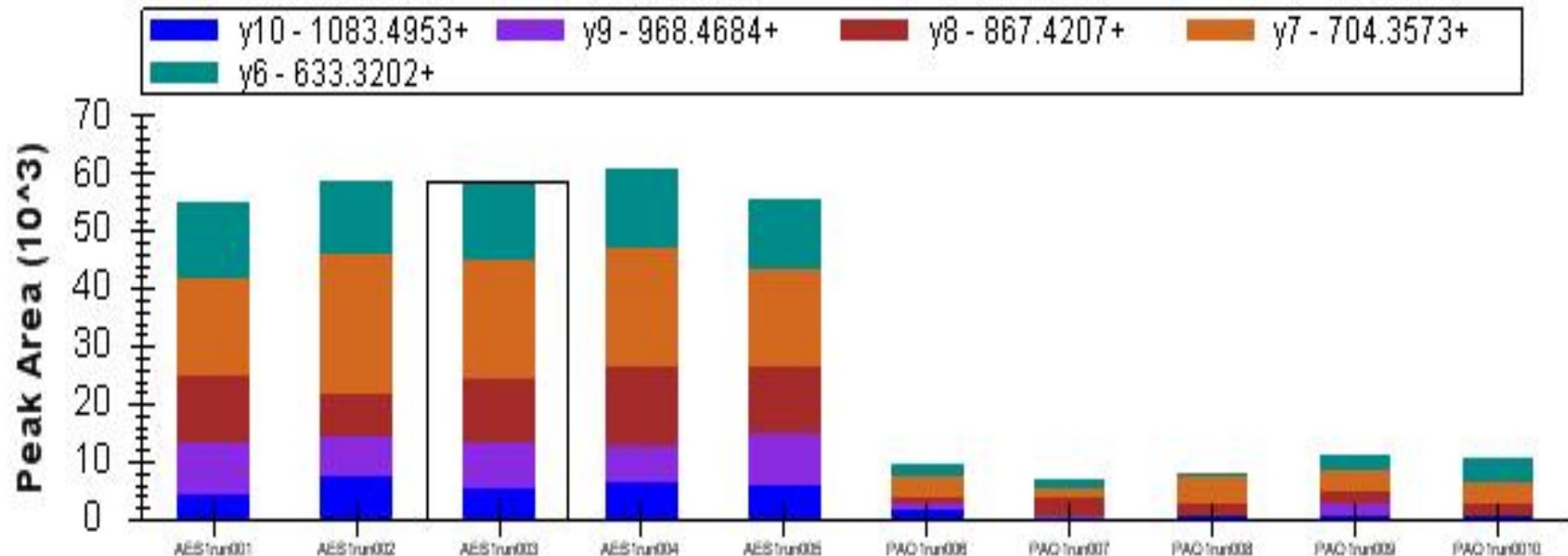
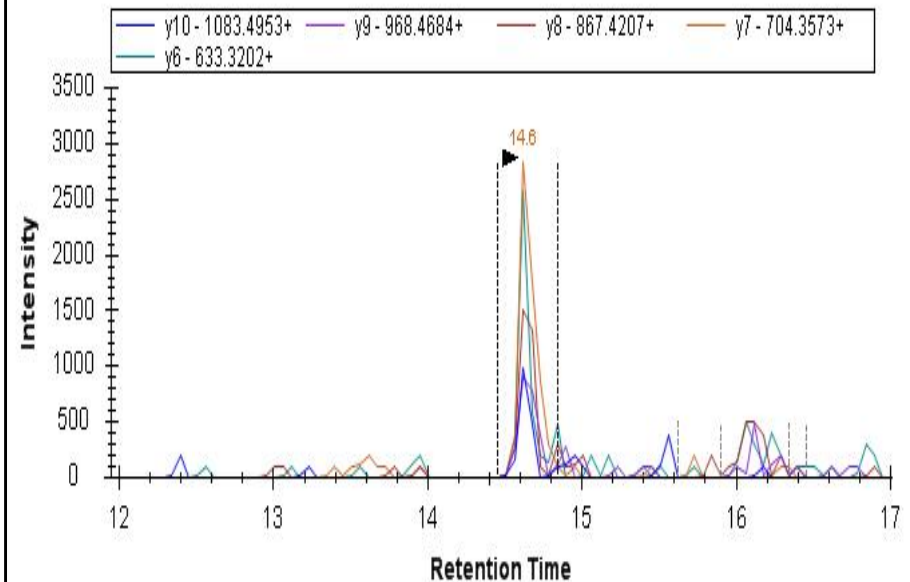
AES-1R Stdev: 2660

PAO1 Stdev: 894

AES-1R CV: 4.5%

PAO1 CV: 8.5%

T-TEST: $p = 2.77 \times 10^{-7}$ Ratio of AES-1R to PAO1: 5.6

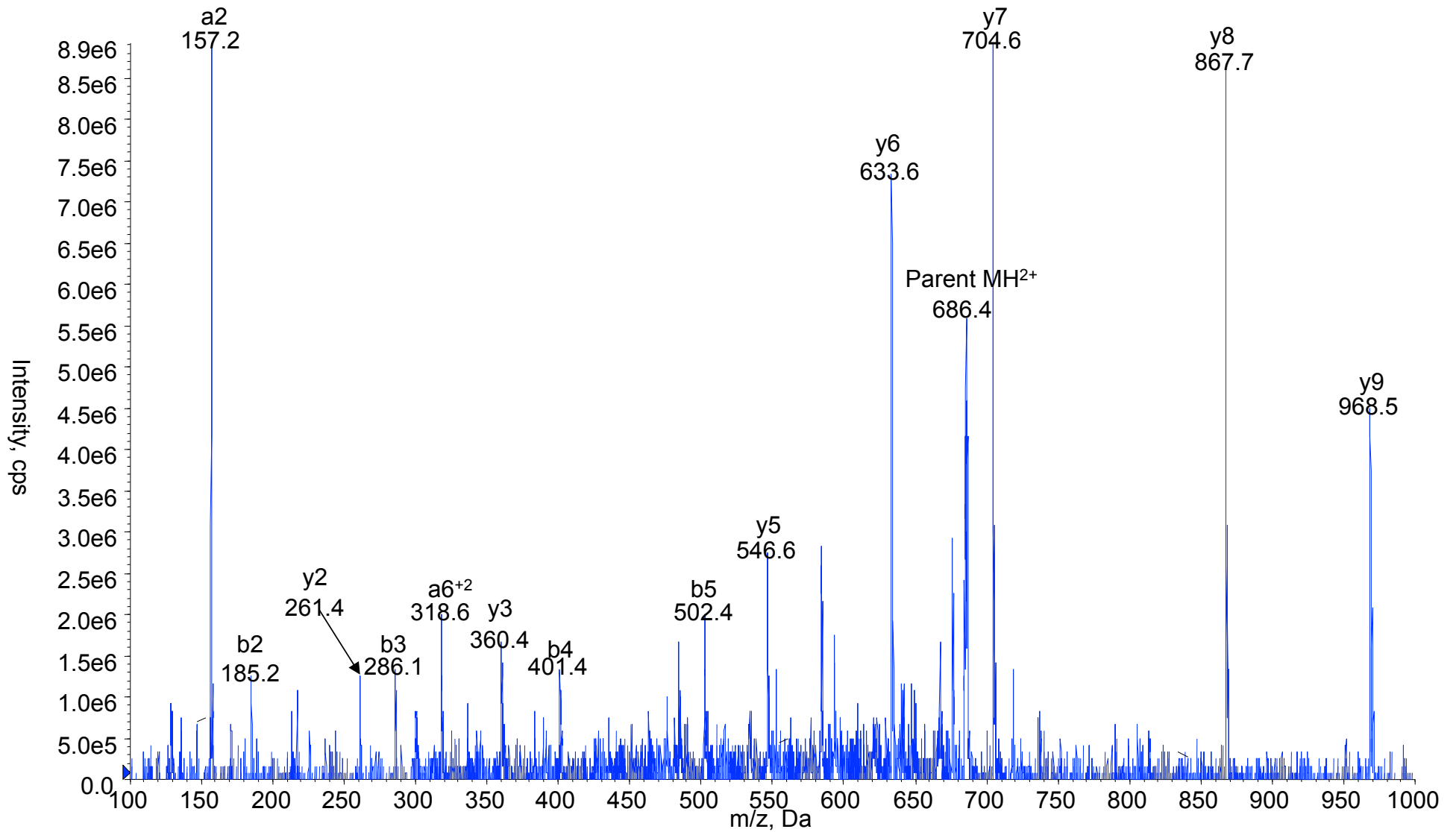


Protein: ABC transporter - PA1342	
Precursor charge: +2	
Precursor m/z: 684.84	
Mascot Ion score: 43	

a2/b2
b3
b4
b5

A I T D T Y A S G E V N K

y9
y8
y7
y6
y5
y3
y2



AES_4384 - Probable Aminopeptidase - PA2939

Peptide Sequence: SPLLVSTPLGLPR

Parent Mass / Charge: 675.41 / +2

Transition list

y11	1165.73032
y10	1052.64626
y9	939.562192
y8	840.493778
y7	753.46175
y6	652.414071

AES-1R mean area: 1452098

PAO1 mean area: 2037753

AES-1R Stdev: 38036

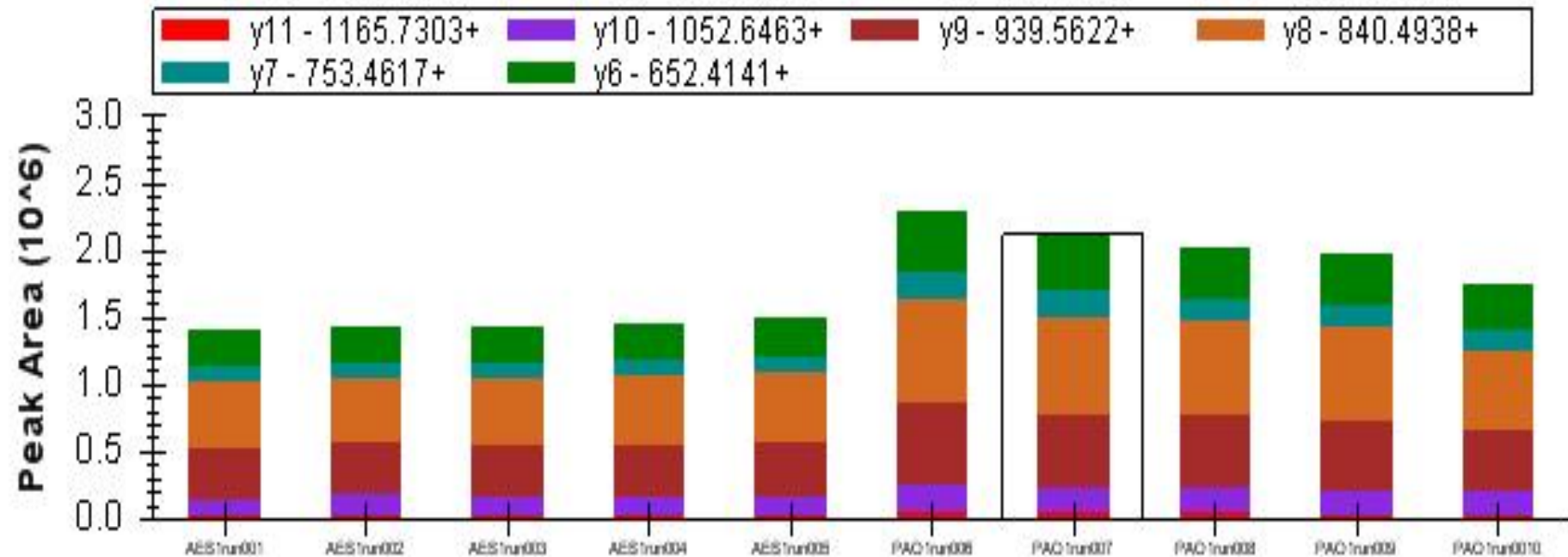
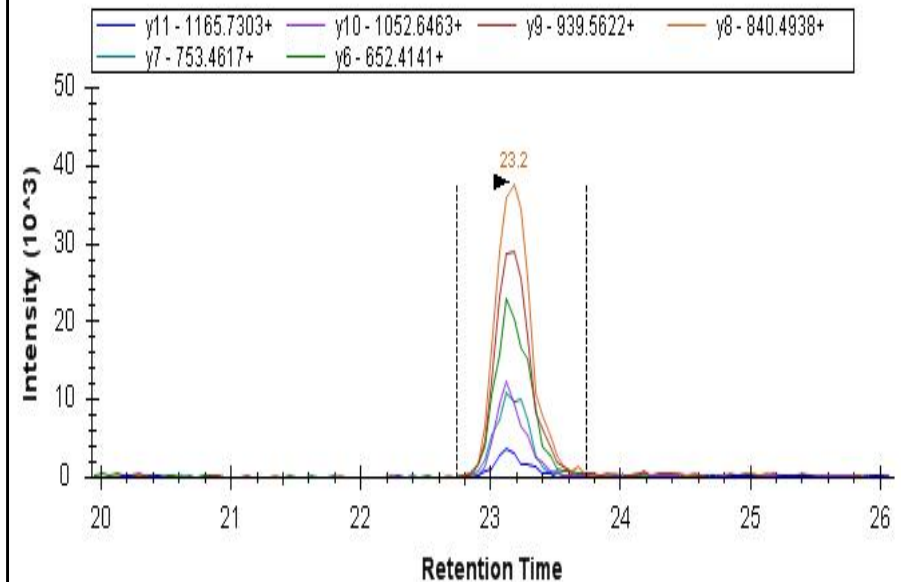
PAO1 Stdev: 197726

AES-1R CV: 2.6%

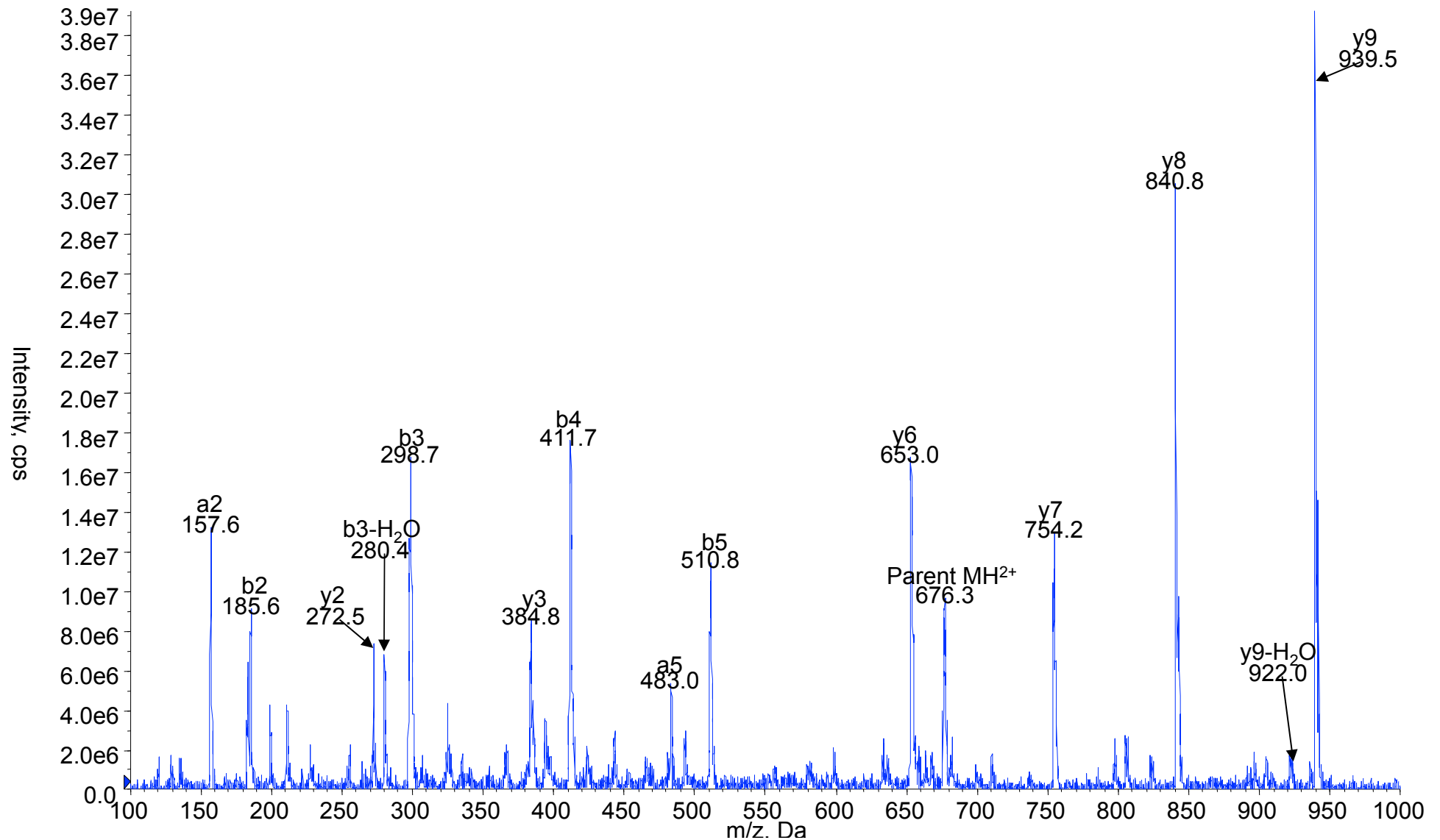
PAO1 CV: 9.7%

T-TEST: $p = 0.002$

Ratio of AES-1R to PAO1: 0.71



Protein: Probable Aminopeptidase-PA2939	a2/b2 b3 b4 a5/b5 y9 y8 y7 y6 y3 y2 S P L L V S T P L G L P R
Precursor charge: +2	
Precursor m/z: 675.41	
Mascot Ion score: 45	



AES_4384 - Probable Aminopeptidase - PA2939

Peptide Sequence: GPGSLSATVPQPVTYEWEK

Parent Mass / Charge: 1023.51 / +2

Transition list

b9	770.404294
y11	1375.68924
y10	1276.62083
y9	1179.56807
y8	1051.50949

AES-1R mean area: 239677

PAO1 mean area: 374539.6

AES-1R Stdev: 9670

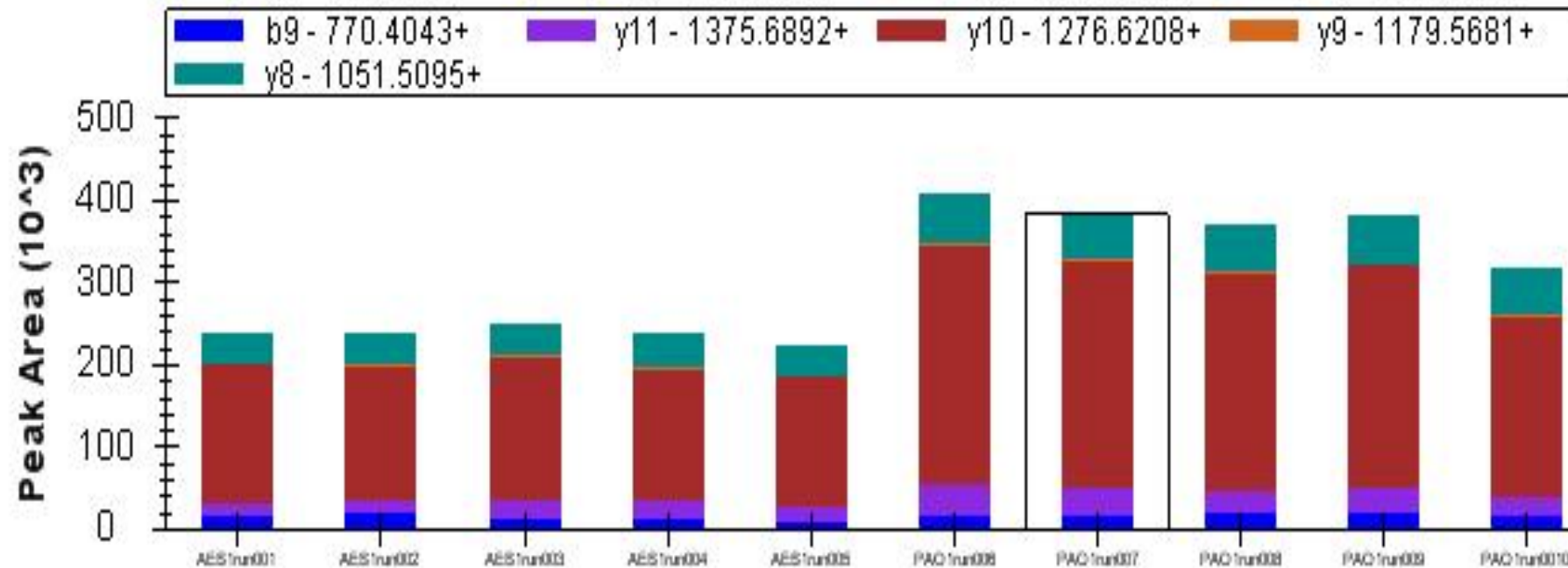
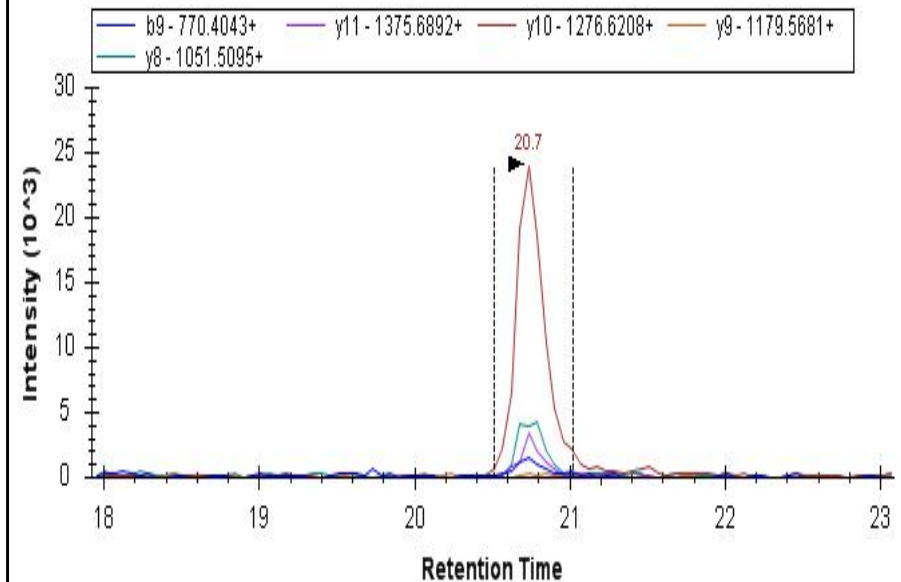
PAO1 Stdev: 34118

AES-1R CV: 4.0%

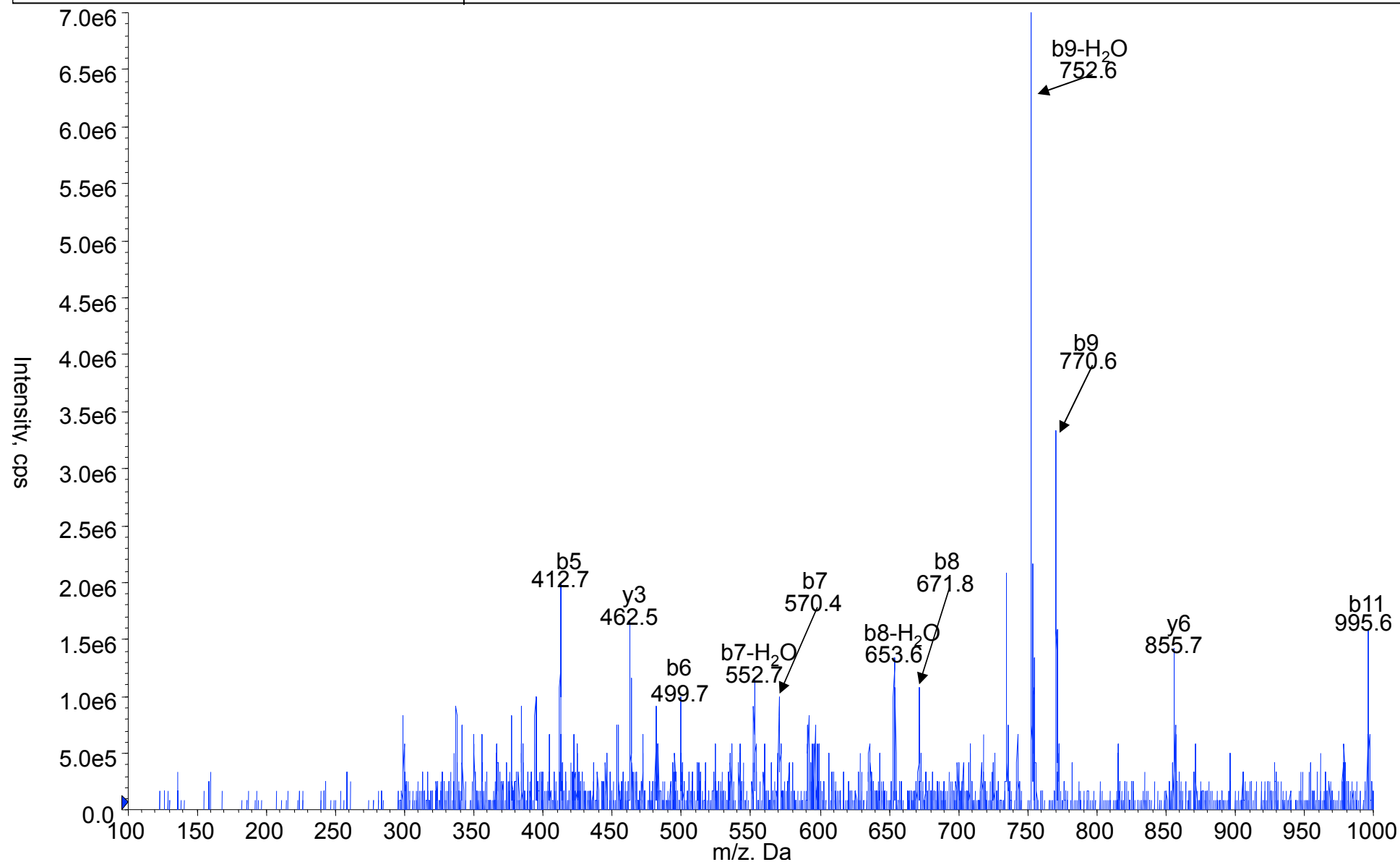
PAO1 CV: 9.1%

T-TEST: $p = 0.0005$

Ratio of AES-1R to PAO1: 0.64



Protein: Probable Aminopeptidase-PA2939	
Precursor charge: +2	
Precursor m/z: 1023.51	G P G S L S A T V P Q P V T Y E W E K
Mascot Ion score: 23	



AES_0809 - PasP Protease - PA0423

Peptide Sequence: NPTATFESTEYK

Parent Mass / Charge: 662.32 / +2

Transition list

y9	1011.49932
y8	940.462203
y7	839.414525
y6	692.346111
y5	563.303518
y11	605.303518

AES-1R mean area: 81323

PAO1 mean area: 36385

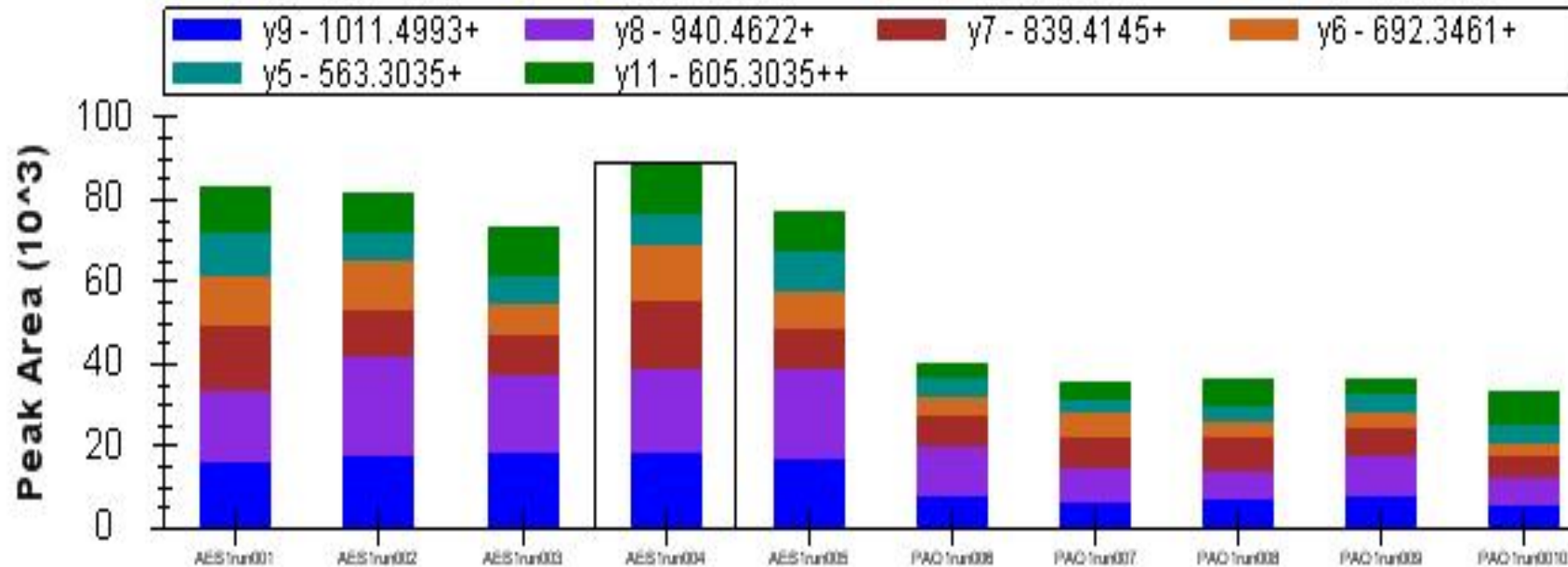
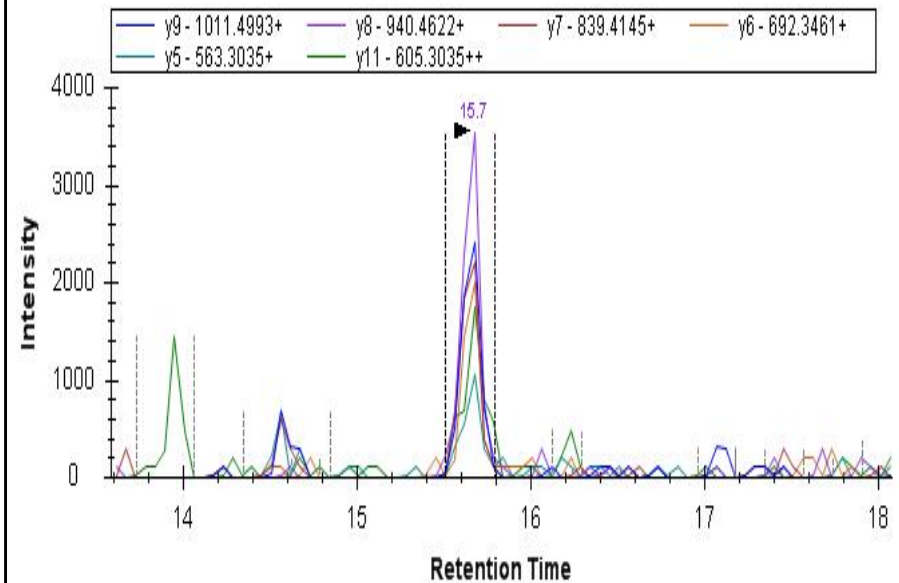
AES-1R Stdev: 5898

PAO1 Stdev: 2612

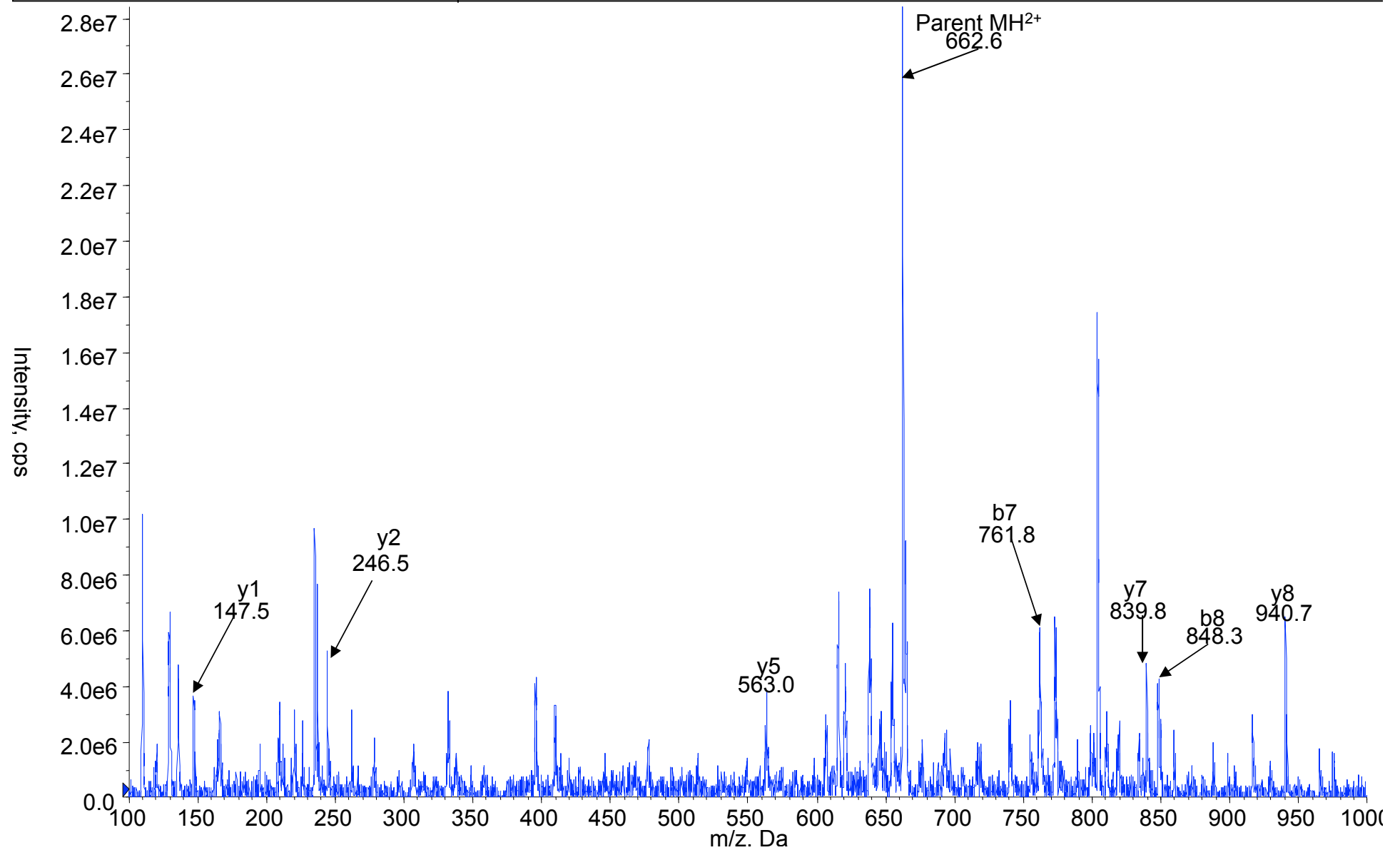
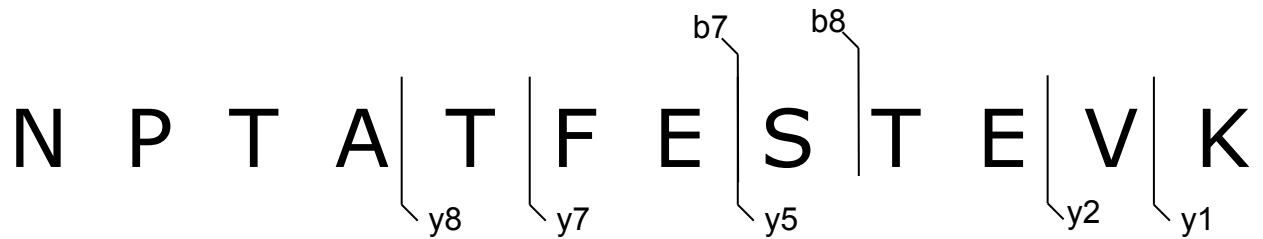
AES-1R CV: 7.3%

PAO1 CV: 7.2%

T-TEST: $p = 9.10 \times 10^{-6}$ Ratio of AES-1R to PAO1: 2.24



Protein: PasP Protease - PA0423	
Precursor charge: +2	
Precursor m/z: 499.24	
Mascot Ion score: 22	



AES_1026 - PvdS-Regulated Endoproteinase - PA4175

Peptide Sequence: GGLYGGPSYCGAPTSQR

Parent Mass / Charge: 864.39 / +2

Transition list

y12	1280.56881
y11	1223.54735
y10	1126.49458
y9	1039.46256
y8	876.399226
y5	588.31

AES-1R mean area: 38044

PAO1 mean area: 39956

AES-1R Stdev: 1383

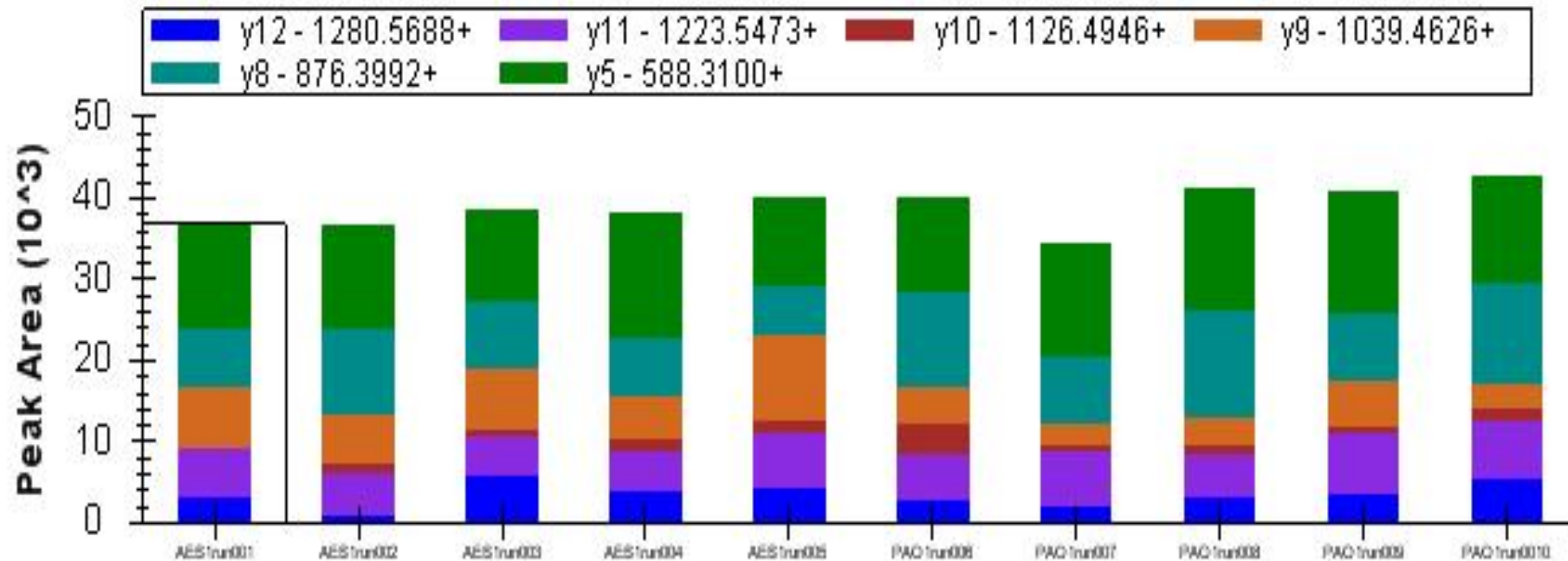
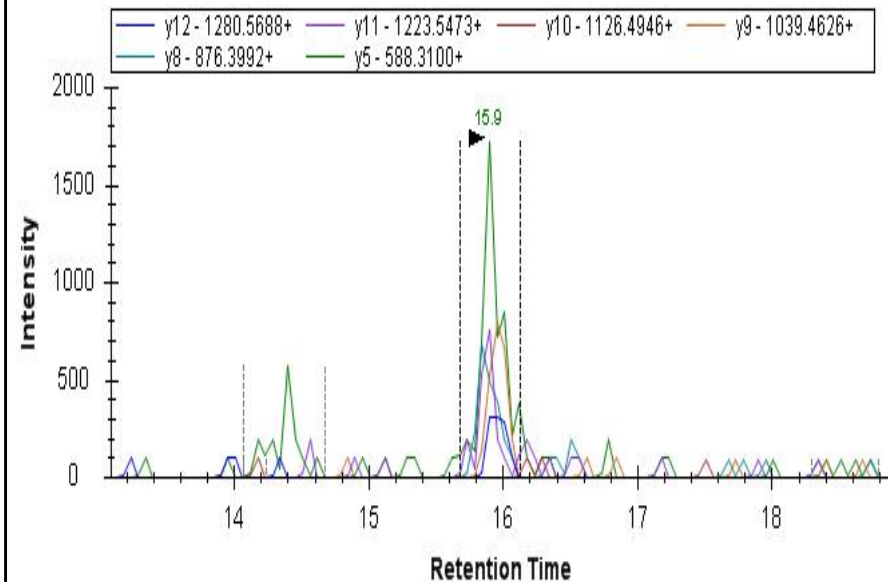
PAO1 Stdev: 3082

AES-1R CV: 3.6%

PAO1 CV: 7.7%

T-TEST: p = 0.25

Ratio of AES-1R to PAO1: 0.95

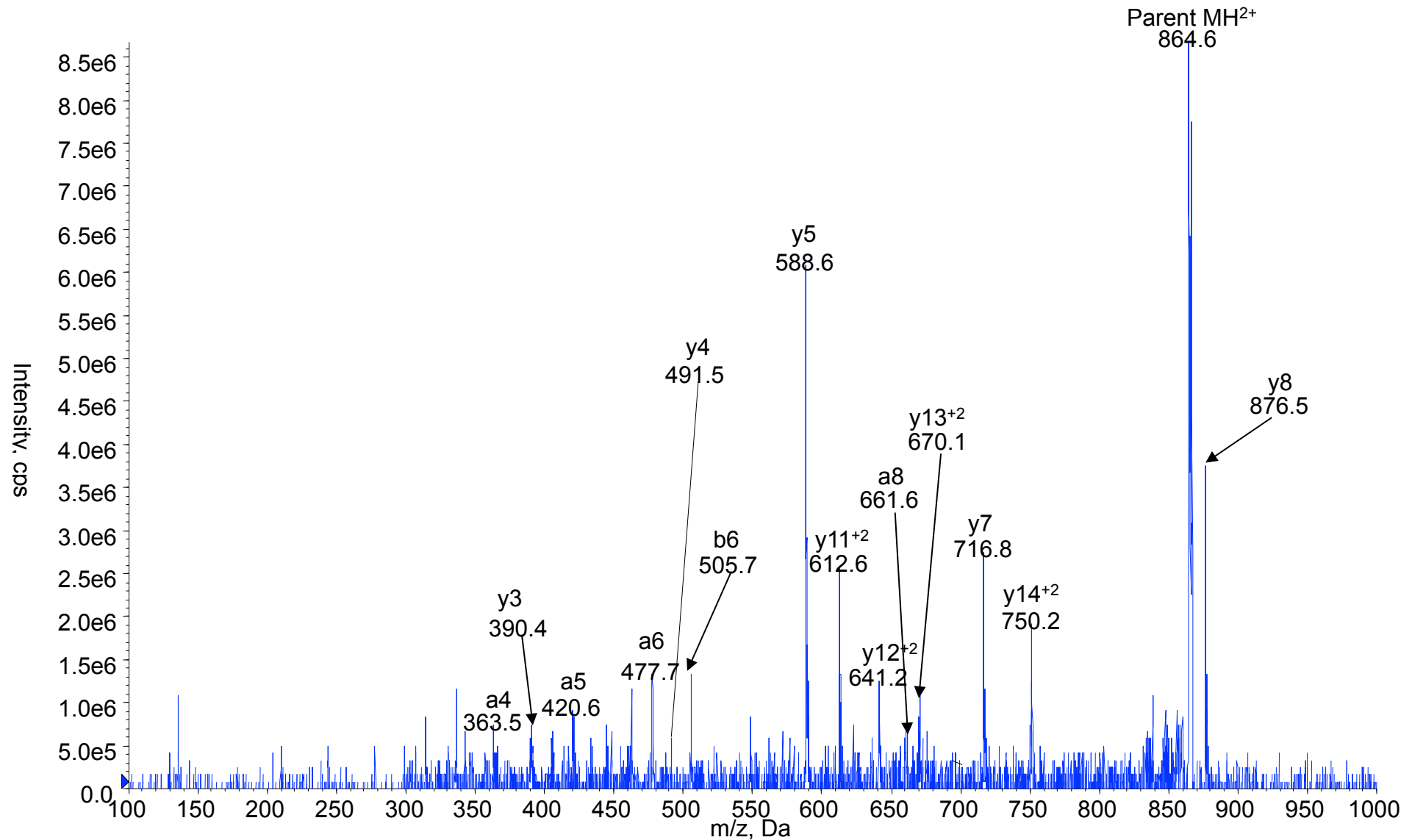


Protein: PrpL - PA4175	
Precursor charge: +2	
Precursor m/z: 864.39	
Mascot Ion score: 28	

a4
a5
a6/b6

G G L Y G G P S Y C G A P T S Q R

y14
y12
y11
y8
y7
y5
y4
y3



AES_1026 - PvdS-Regulated Endoproteinase - PA4175

Peptide Sequence: NDYFSDFGVYSQISR

Parent Mass / Charge: 942.92 / +2

Transition list

y12	1345.63827
y11	1258.60624
y10	1143.5793
y9	996.510885
y8	909.478856
y7	852.457393

AES-1R mean area: 156127

PAO1 mean area: 143595

AES-1R Stdev: 3921

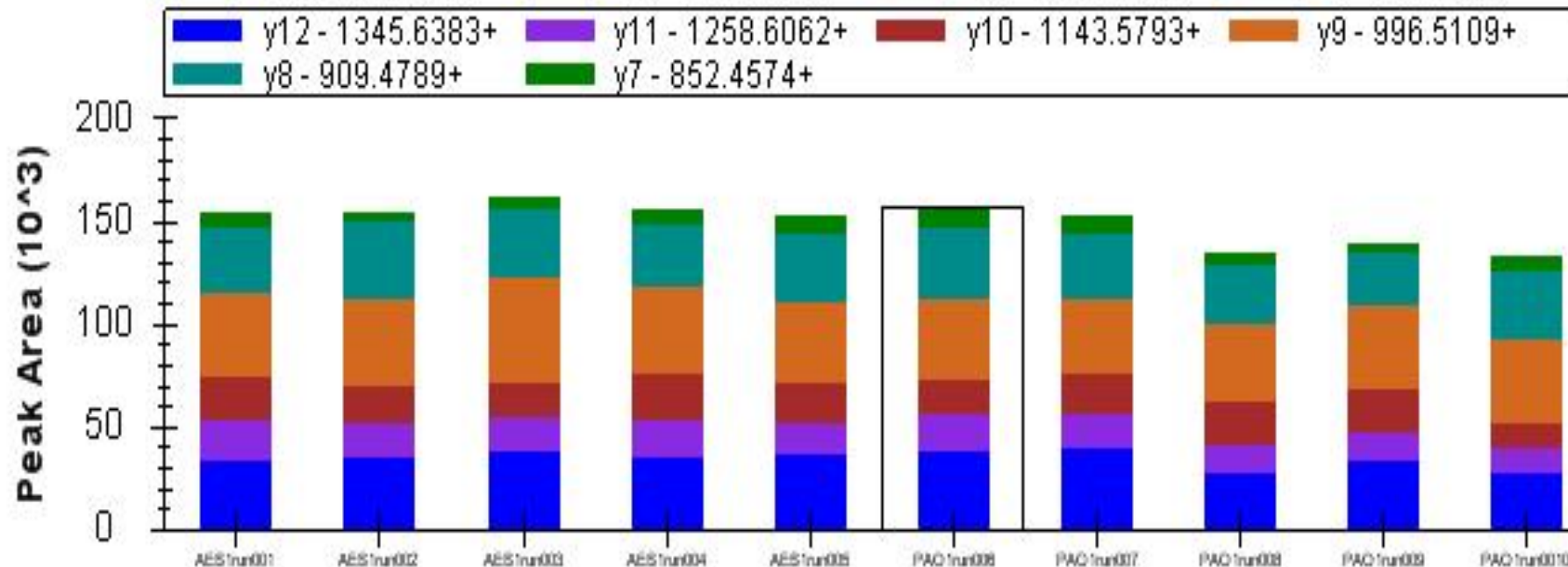
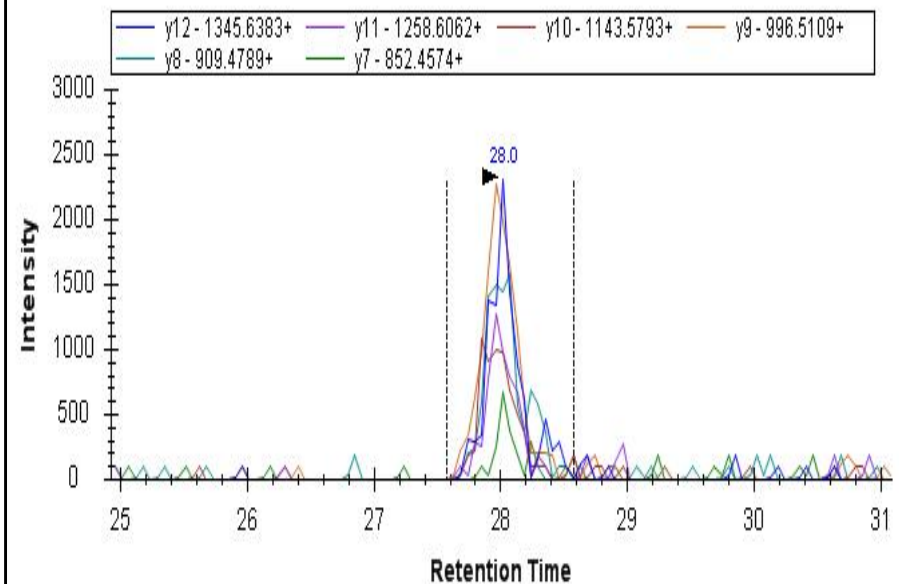
PAO1 Stdev: 11031

AES-1R CV: 2.5%

PAO1 CV: 7.7%

T-TEST: $p = 0.06$

Ratio of AES-1R to PAO1: 1.09



Protein: PrpL - PA4175	
Precursor charge: +2	
Precursor m/z: 942.92	
Mascot Ion score: 62	

b3
a4/b4
b6
b7

N D Y F S D F S G V Y S Q I S R

y9
y8
y7
y6
y5
y4
y3

