Supporting information to:

Mutational studies of the mersacidin leader reveal the function of its unique two-step leader processing mechanism.

Jakob H. Viel, Oscar P. Kuipers*

Department of Molecular Genetics, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

***Correspondence:**
Oscar P. Kuipers
o.p.kuipers@rug.nl

Keywords: Mersacidin, RiPP, lanthipeptide, leader, heterologous expression, *E. coli,* mutation

Contents

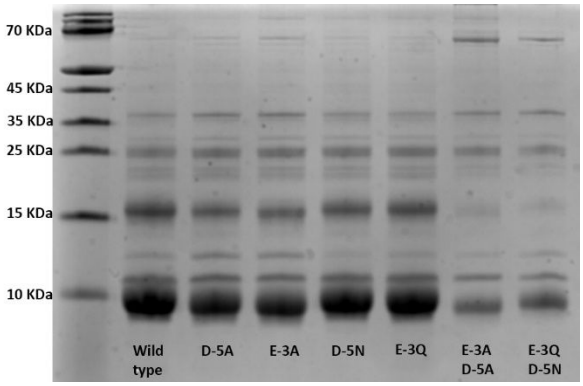
S1. Full amino acid sequence of all mutants

		Sequence
a	**Wild type (His6-MrsA)**	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGDMEAACTFTL PGGGGVCTLTSECIC
b	pACYC E-3A + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGDMAAACTFTL PGGGGVCTLTSECIC
c	pACYC D-5A + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGAMEAACTFTL PGGGGVCTLTSECIC
d	pACYC E-3Q + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGDMQAACTFTL PGGGGVCTLTSECIC
e	pACYC D-5N + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGNMEAACTFTL PGGGGVCTLTSECIC
f	pACYC E-3A D-5A + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGAMAAACTFTL PGGGGVCTLTSECIC
g	pACYC E-3Q N-5A + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGNMQAACTFTL PGGGGVCTLTSECIC
h	pACYC E-3K + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGDMKAACTFTL PGGGGVCTLTSECIC
i	pACYC D-5K + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGKMEAACTFTL PGGGGVCTLTSECIC
j	pACYC AGDMEA + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAAGDMEACTFTL PGGGGVCTLTSECIC
k	pACYC DMEAAA + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGADMEAAACTFTL PGGGGVCTLTSECIC
l	pACYC ΔDMEA + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGACTFTL PGGGGVCTLTSECIC
m	pACYC ΔDMEAA + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGCTFTL PGGGGVCTLTSECIC
n	pACYC ΔGDMEAA + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGACTFTL PGGGGVCTLTSECIC
o	pACYC ΔA-1 + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGDMEACTFTL PGGGGVCTLTSECIC
p	pACYC ΔG-6 + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGADMEAACTFTL PGGGGVCTLTSECIC
q	pACYC ΔK-11 + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDLVGAGDMEAACTFTL PGGGGVCTLTSECIC
r	pACYC ΔE-16 + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKAQMDKLVGAGDMEAACTFTL PGGGGVCTLTSECIC
s	pACYC ΔF-21 + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPSELKEAQMDKLVGAGDMEAACTFTL PGGGGVCTLTSECIC
t	pACYC K-11A + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDALVGAGDMEAACTFTL PGGGGVCTLTSECIC
u	pACYC E-16A + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPFSELKAAQMDKLVGAGDMEAACTFTL PGGGGVCTLTSECIC
v	pACYC F-21A + MrsM	MHHHHHHGSQEAIIRSWKDPFSRENSTQNPAGNPASELKEAQMDKLVGAGDMEAACTFTL PGGGGVCTLTSECIC

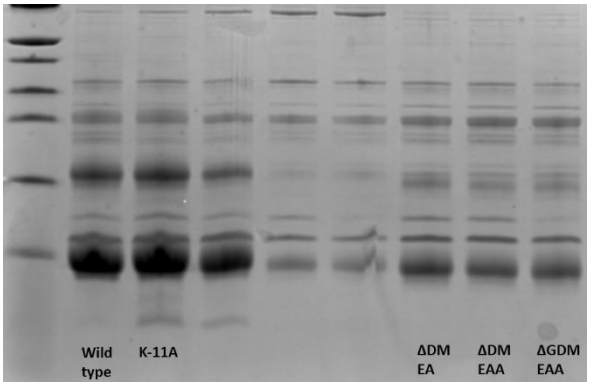
S2. Yield determination by Tricine-Page

The freeze-dried C-18 elution fraction of each peptide was dissolved in 150 µL of Milli-Q water, 4 µL of this solution was run on gel. Thus, effectively 8 mL of expression culture was run per well in case of 100 % purification efficiency. Non annotated wells contain peptides not discussed in this study.

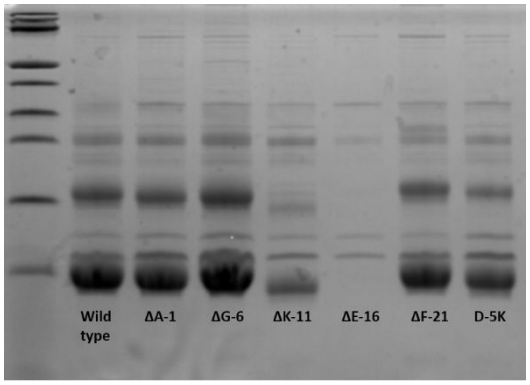
Gel 1



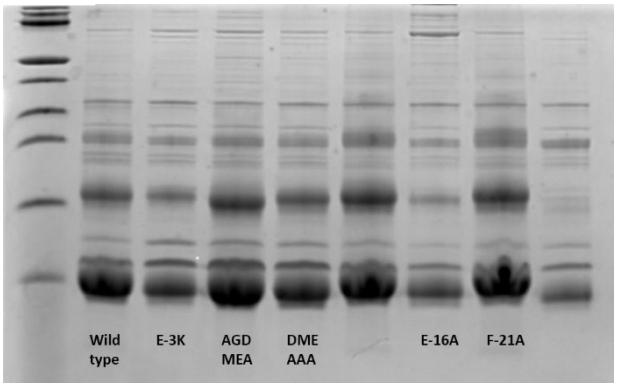
Gel 2



Gel 3

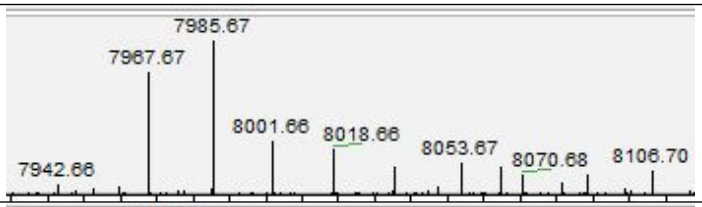
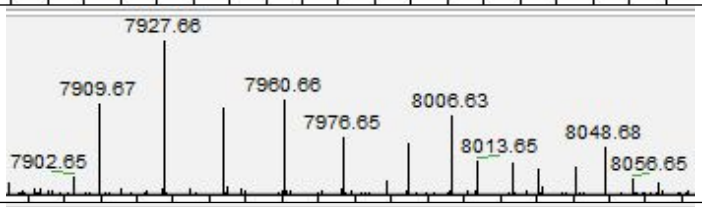
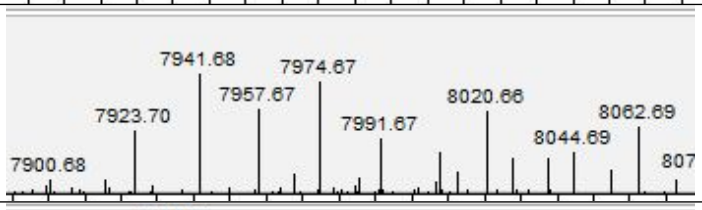
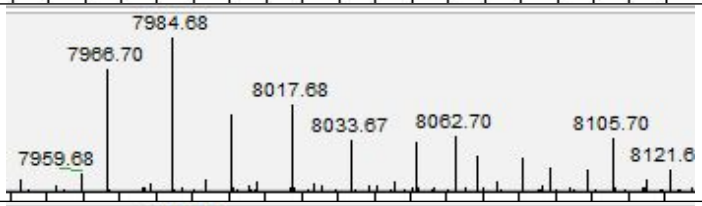
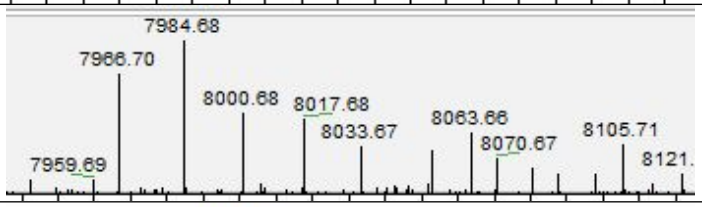
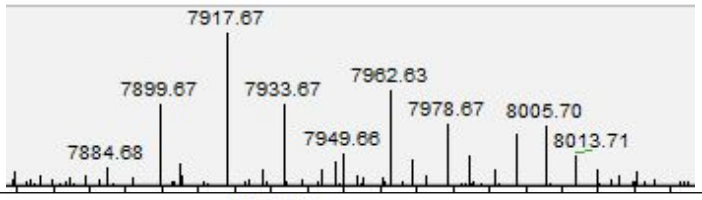
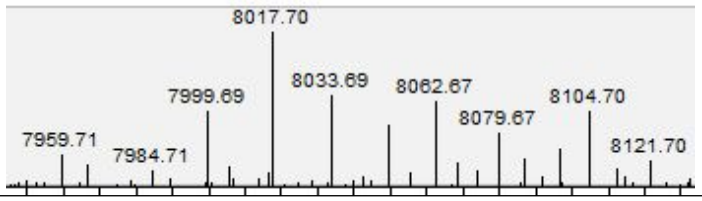


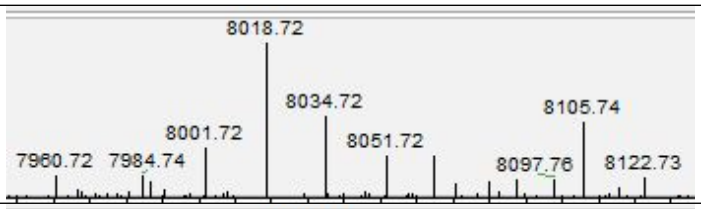
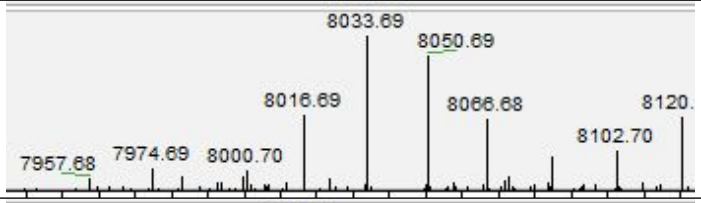
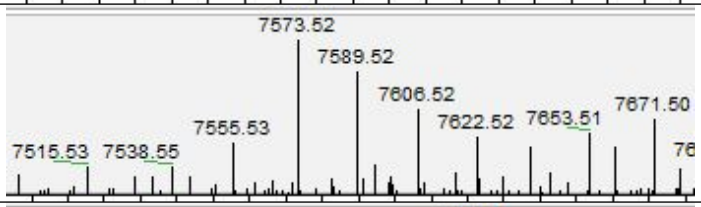
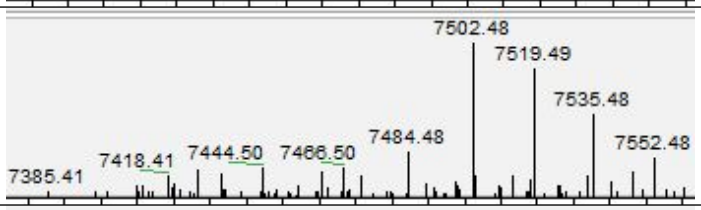
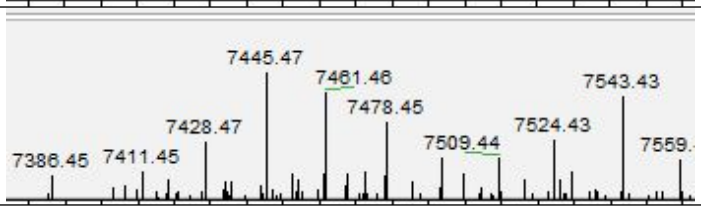
Gel 4

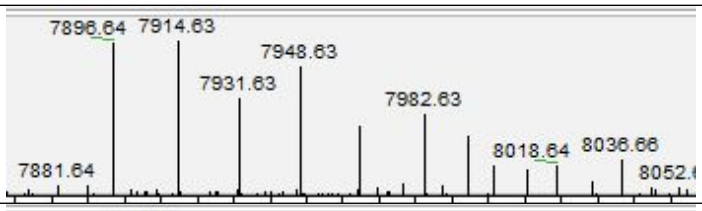
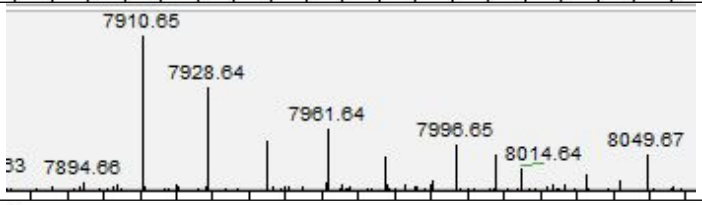
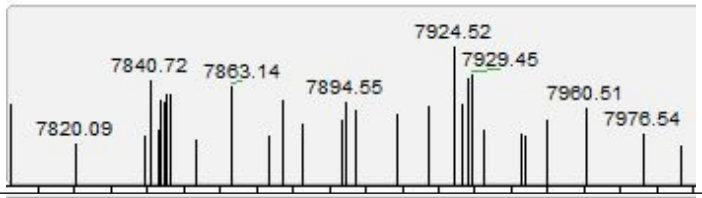
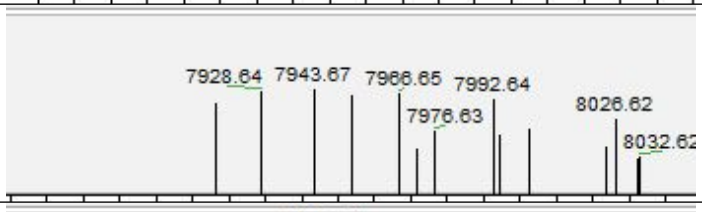
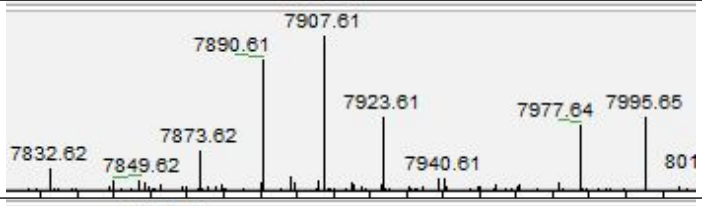
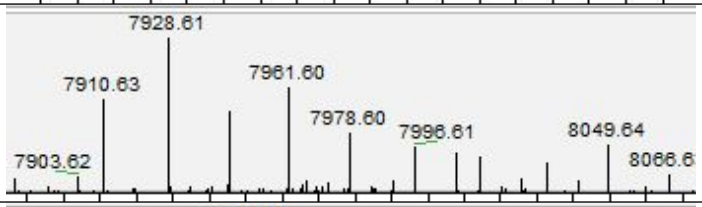
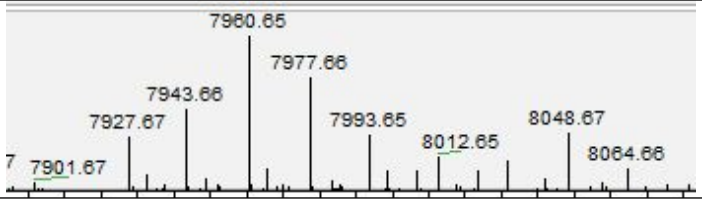


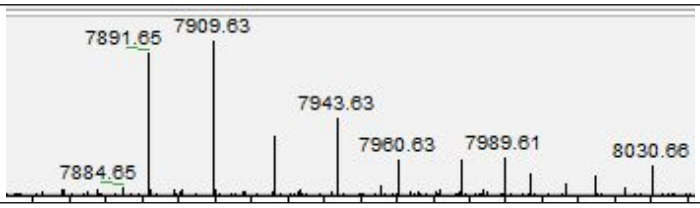
S3. LC-MS analysis of mutants

All masses are the MrsD decarboxylated masses

	Mutant	Spectrum	-H_2O	Mono-isotopic mass (Da)	
a	Wild type		0	8057,70	
			1	8039,69	
			2	8021,68	
			3	8003,67	
			4	7985,66	
			5	7967,65	
b	D-5A		0	7999,69	
			1	7981,68	
			2	7963,67	
			3	7945,66	
			4	7927,65	
			5	7909,64	
c	E-3A		0	8013,71	
			1	7995,70	
			2	7977,69	
			3	7959,68	
			4	7941,67	
			5	7923,66	
d	D-5N		0	8056,71	
			1	8038,70	
			2	8020,69	
			3	8002,68	
			4	7984,67	
			5	7966,66	
e	E-3Q		0	8056,71	
			1	8038,70	
			2	8020,69	
			3	8002,68	
			4	7984,67	
			5	7966,66	
f	E-3A D5A		0	7955,70	Mostly -3 H_2O - $2H^+$, with trace amounts of -4 H_2O.
			1	7937,69	
			2	7919,68	
			3	7901,67	
			4	7883,66	
			5	7865,65	
g	E-3Q D5N		0	8055,73	Mostly -3 H_2O - $2H^+$, with trace amounts of -4 H_2O.
			1	8037,72	
			2	8019,71	
			3	8001,70	
			4	7983,69	
			5	7965,68	

	Mutant	Spectrum	- H_2O	Mono-isotopic mass (Da)	
h	E-3K		0	8056,75	-5 H_2O undetectable, but antimicrobial activity shows its presence
			1	8038,74	
			2	8020,73	
			3	8002,72	
			4	7984,71	
			5	7966,70	
i	D-5K		0	8070,77	-5 H_2O undetectable, but antimicrobial activity shows its presence
			1	8052,76	
			2	8034,75	
			3	8016,73	
			4	7998,72	
			5	7980,71	
j	AGDMEA		0	8057,70	Fully dehydrated, but at least one ring is not formed.
			1	8039,69	
			2	8021,68	
			3	8003,67	
			4	7985,66	
			5	7967,65	
k	DMEAAA		0	8071,71	At most -3 H_2O, with trace amounts of -4 H_2O.
			1	8053,70	
			2	8035,69	
			3	8017,68	
			4	7999,67	
			5	7981,66	
l	ΔDMEA		0	7611,55	Mostly -3 H_2O - $2H^+$, with trace amounts of -4 H_2O.
			1	7593,54	
			2	7575,53	
			3	7557,52	
			4	7539,51	
			5	7521,50	
m	ΔDMEAA		0	7540,51	Mostly -3 H_2O - $2H^+$
			1	7522,50	
			2	7504,49	
			3	7486,48	
			4	7468,47	
			5	7450,46	
n	ΔGDMEAA		0	7483,49	Mostly -3 H_2O - $2H^+$, with trace amounts of -4 H_2O.
			1	7465,48	
			2	7447,47	
			3	7429,46	
			4	7411,45	
			5	7393,44	

	Mutant	Spectrum	-H_2O	Mono-isotopic mass (Da)	
o	ΔA-1		0	7986,66	Fully dehydrated, but at least one ring is not formed.
			1	7968,65	
			2	7950,64	
			3	7932,63	
			4	7914,62	
			5	7896,61	
p	ΔG-6		0	8000,68	Production and modification as good as wild type
			1	7982,67	
			2	7964,66	
			3	7946,65	
			4	7928,63	
			5	7910,62	
q	ΔK-11		0	7929,60	Poor production and modification efficiency
			1	7911,59	
			2	7893,58	
			3	7875,57	
			4	7857,56	
			5	7839,55	
r	ΔE-16		0	7928,66	Very poor production. No modification
			1	7910,65	
			2	7892,63	
			3	7874,62	
			4	7856,61	
			5	7838,60	
s	ΔF-21		0	7910,63	Poor production and modification efficiency
			1	7892,62	
			2	7874,61	
			3	7856,60	
			4	7838,59	
			5	7820,58	
t	K-11A		0	8000,64	Production and modification almost as good as wild type
			1	7982,63	
			2	7964,62	
			3	7946,61	
			4	7928,60	
			5	7910,59	
u	E-16A		0	7999,69	-5 H_2O undetectable, but antimicrobial activity shows its presence
			1	7981,68	
			2	7963,67	
			3	7945,66	
			4	7927,65	
			5	7909,64	

	Mutant	Spectrum	-H_2O	Mono-isotopic mass (Da)	
V	F-21A		0	7981,67	
			1	7963,66	
			2	7945,65	
			3	7927,64	
			4	7909,62	
			5	7891,61	

S4. Antimicrobial activity tests of all mutants

To assure maximum resolution of the activity tests, all mutants were first applied in high amounts, applying yields from 16 mL of culture digested by AprE-His (plate 1-3). As a control, an AprE-His digested mixed yield from three wild type expressions was applied in an equivalent of 4 mL of culture on all plates. Also 225 ng of nisin was spotted as a positive control. Next, the mutants showing an activity high enough to be limited by diffusion were tested again (plate 4), this time using half the amount (8 mL of culture equivalent). Finally, the compounds in plate five were spotted in equal amounts as the control, namely 4 mL equivalent of expression culture.

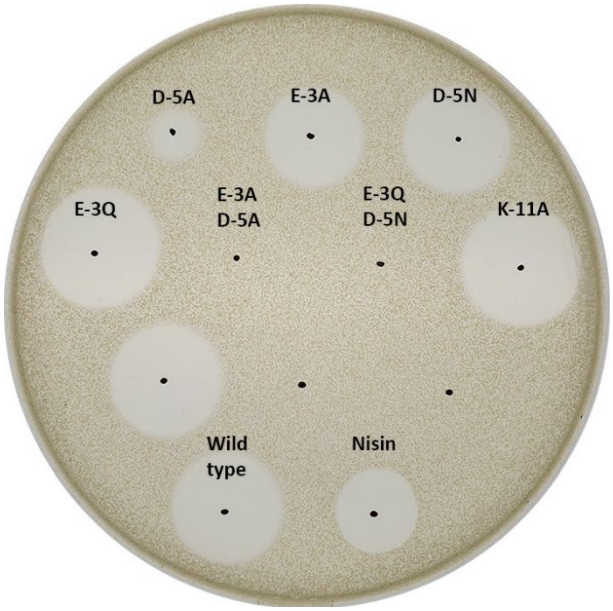


Plate 1: Mutants spotted **4:1** compared to wild type control.

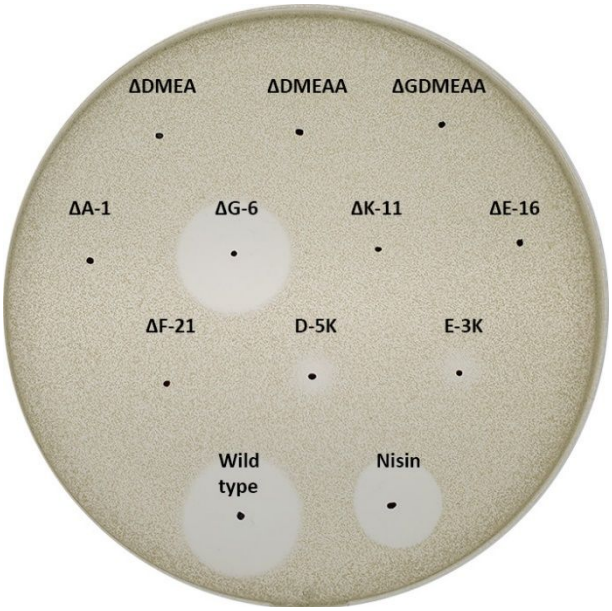


Plate 2: Mutants spotted **4:1** compared to wild type control.

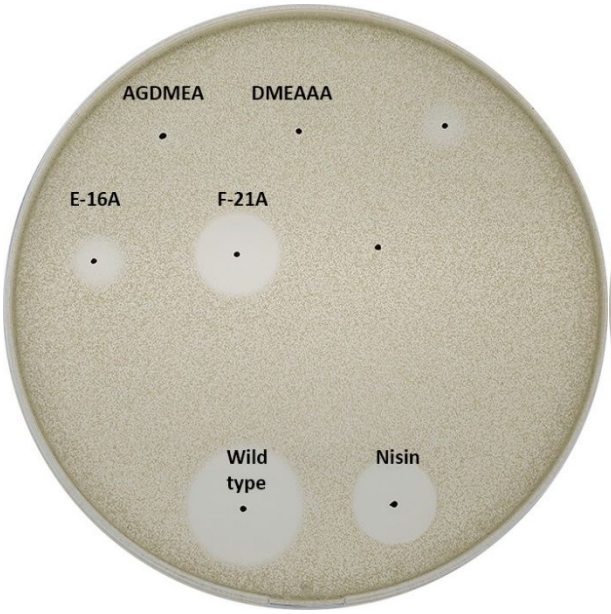


Plate 3: Mutants spotted **4:1** compared to wild type control.

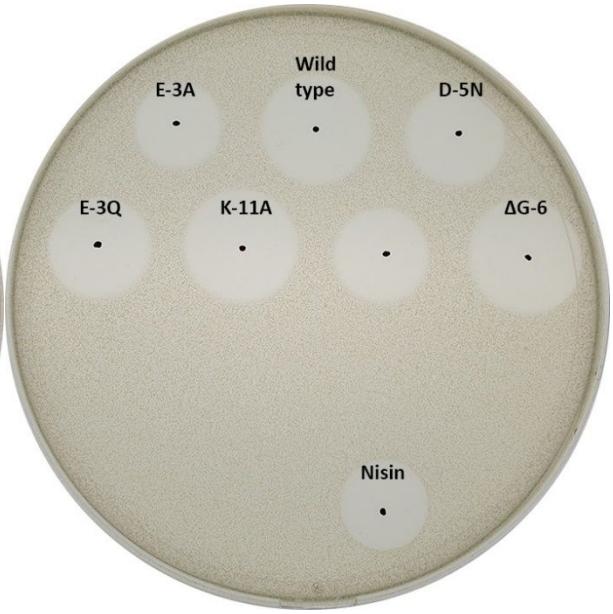


Plate 4: Mutants spotted **2:1** compared to wild type control.

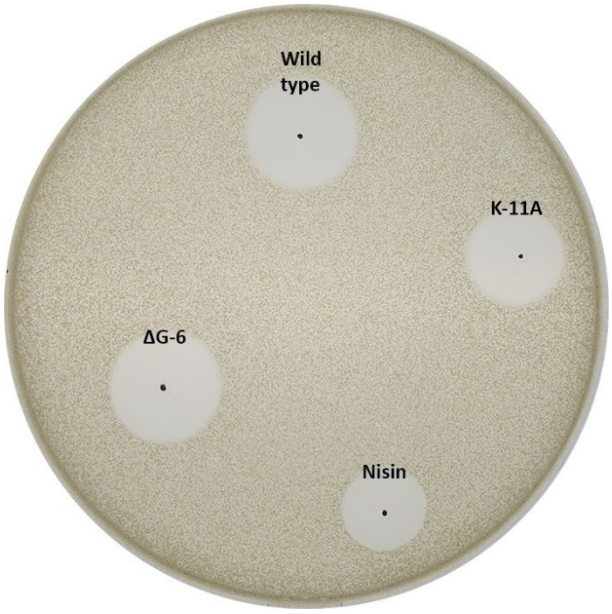


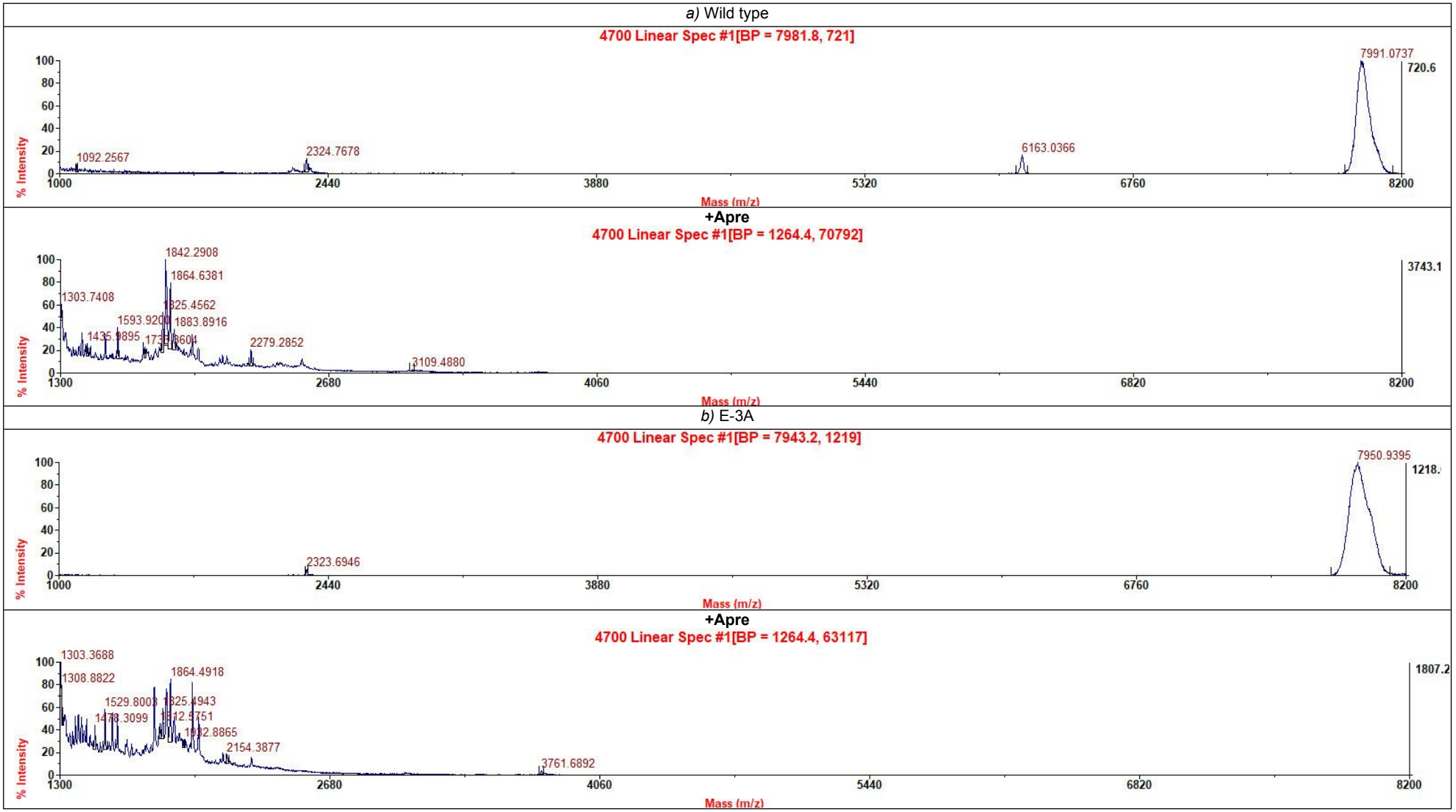
Plate 5: Mutants spotted **1:1** compared to wild type control.

To determine the relative activity, the area of each of the tested mutants was compared with that of the wild type control, which was set to 1. For mutants tested in more than one volume, the halo from the smallest spotted volume was used. The difference in surface area compared to the wild type control was directly translated to a fraction of the wild type activity. Based on this fraction, an activity rating of 0 to 5 was given based on an exponential scale best representing the differences in activity between the tested mutants (See table below).

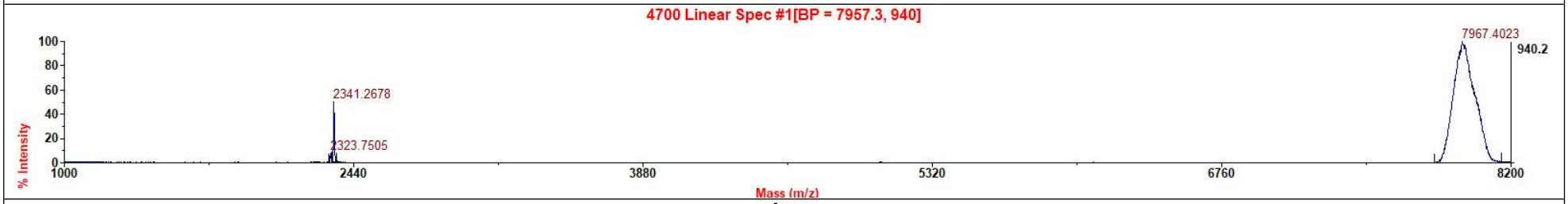
Relative antimicrobial activity

	Mutant	Plate	Halo Diameter (cm)	Halo Area	Amount Ratio	Activity per ratio	Fraction of activity	>0	>0,09375	>0,1875	>0,375	>0,75
c	D-5A	1	1,98	3,08	4	0,77	0,05	+			(of wildtype activity)	
f	E-3A D-5A	1	0,00	0,00	4	0,00	0,00					
g	E-3Q N-5A	1	0,00	0,00	4	0,00	0,00					
a	***wild type***	1	4,55	16,26	1	16,26	1,00					
l	ΔDMEA	2	0,00	0,00	4	0,00	0,00					
m	ΔDMEAA	2	0,00	0,00	4	0,00	0,00					
n	ΔGDMEAA	2	0,00	0,00	4	0,00	0,00					
o	ΔA-1	2	0,00	0,00	4	0,00	0,00					
q	ΔK-11	2	0,00	0,00	4	0,00	0,00					
r	ΔE-16	2	0,00	0,00	4	0,00	0,00					
s	ΔF-21	2	0,00	0,00	4	0,00	0,00					
i	D-5K	2	1,10	0,95	4	0,24	0,01	+				
h	E-3K	2	0,75	0,44	4	0,11	0,01	+				
a	***wild type***	2	4,91	18,93	1	18,93	1,00					
j	AGDMEA	3	0,00	0,00	4	0,00	0,00					
k	DMEAAA	3	0,00	0,00	4	0,00	0,00					
u	E-16A	3	1,50	1,77	4	0,44	0,03	+				
v	F-21A	3	3,26	8,35	4	2,09	0,12	+	+			
a	***wild type***	3	4,70	17,35	1	17,35	1,00					
b	E-3A	4	3,46	9,40	2	4,70	0,30	+	+	+		
e	D-5N	4	3,85	11,64	2	5,82	0,37	+	+	+		
d	E-3Q	4	3,89	11,88	2	5,94	0,38	+	+	+	+	
a	***wild type***	4	4,48	15,76	1	15,76	1,00					
t	K-11A	5	3,58	10,07	1	10,07	0,70	+	+	+	+	
p	ΔG-6	5	4,35	14,86	1	14,86	1,03	+	+	+	+	+
a	***wild type***	5	4,28	14,39	1	14,39	1,00					

S5. Spectra of AprE cleaved mutants



c) D-5A



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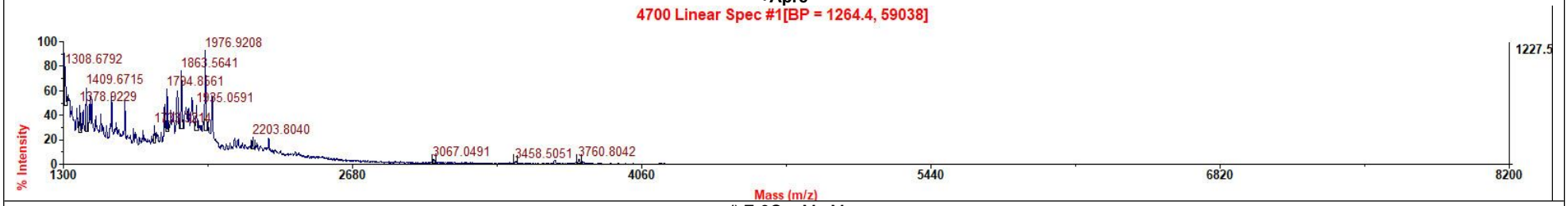
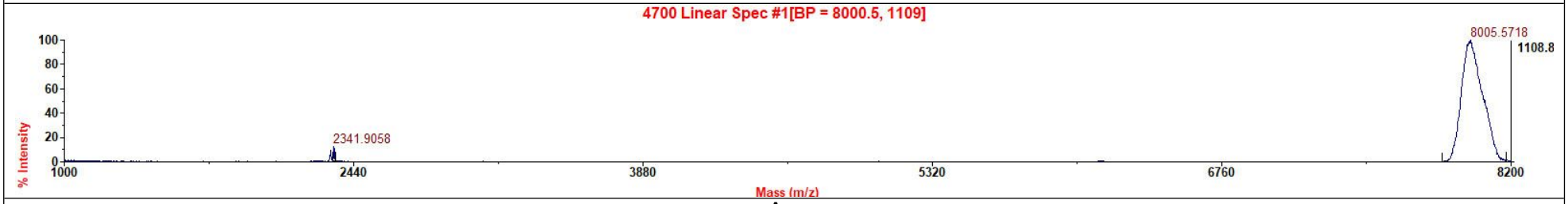


d) E-3Q + MrsM



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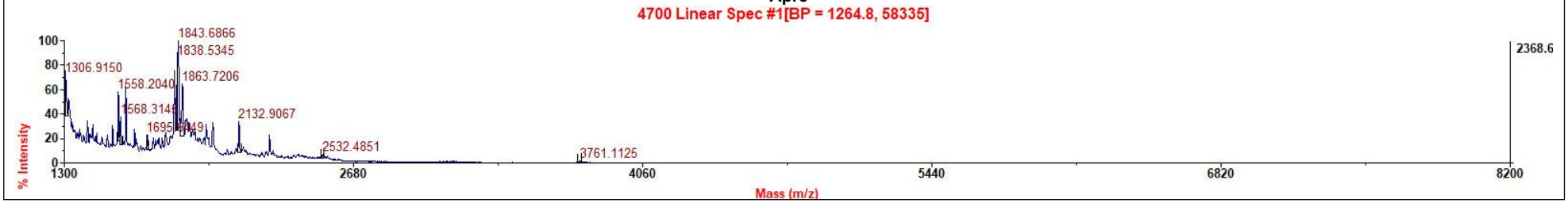
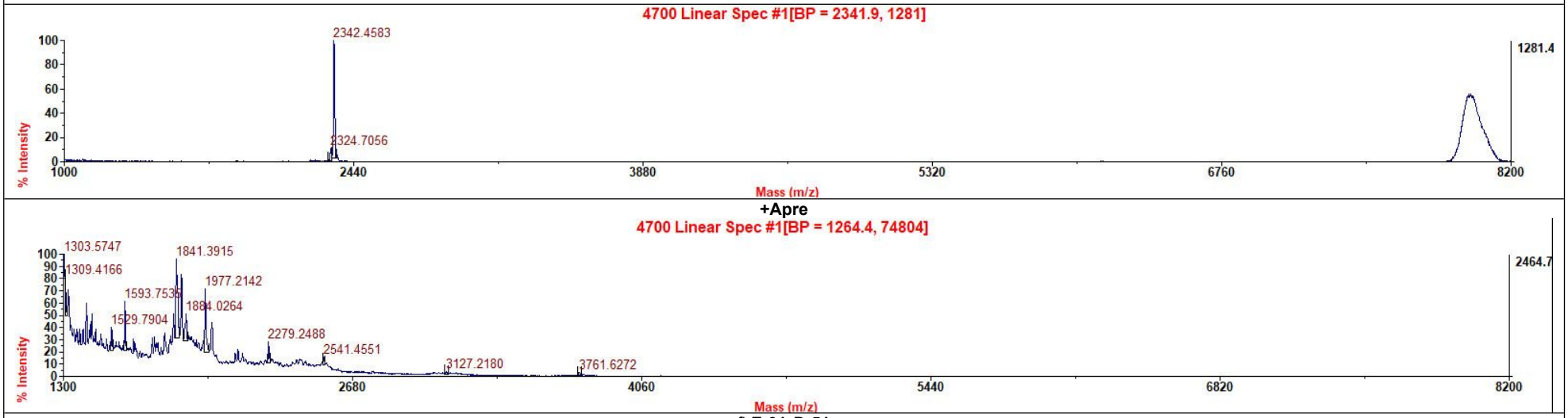
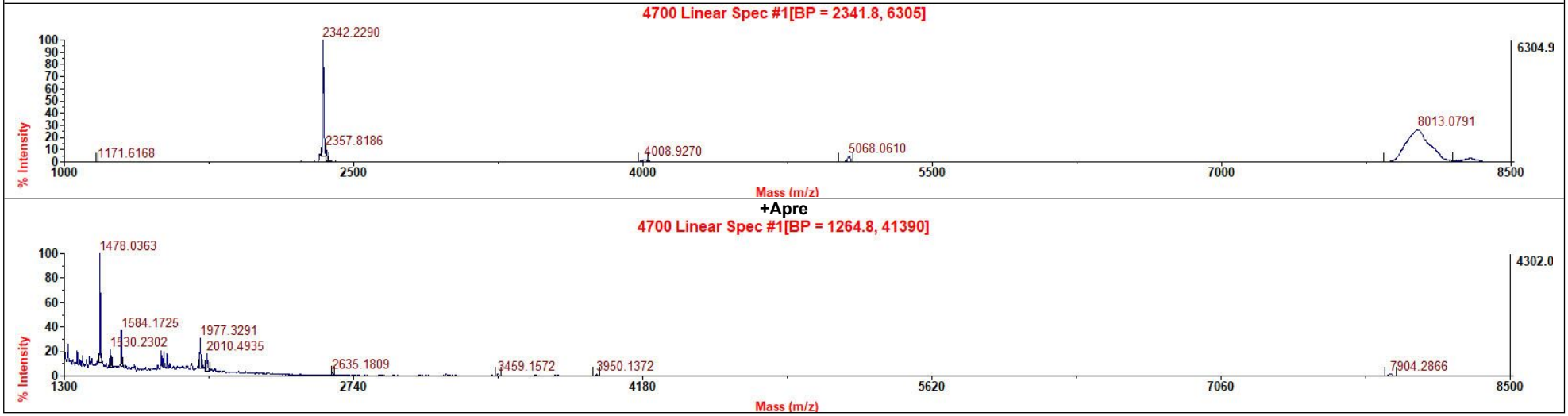


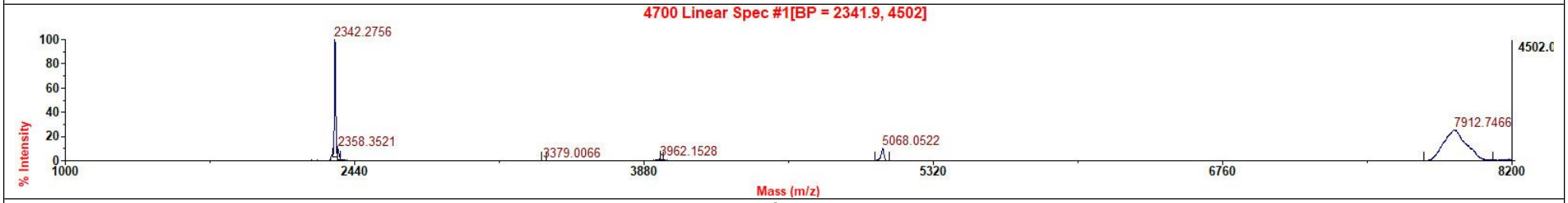
e) D-5N



f) E-3A D-5A



g) E-3Q N-5A



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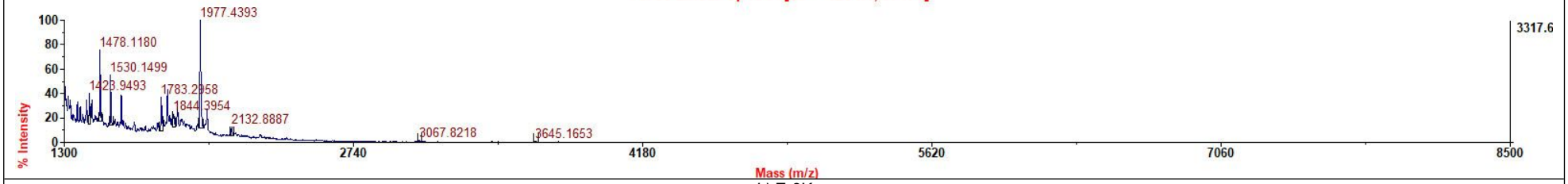
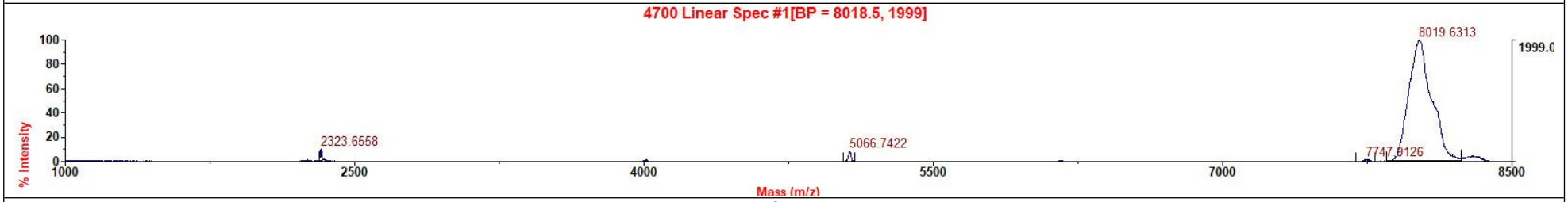


h) E-3K



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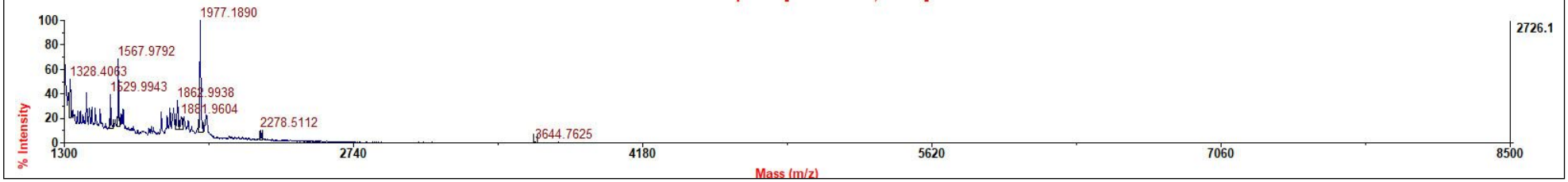
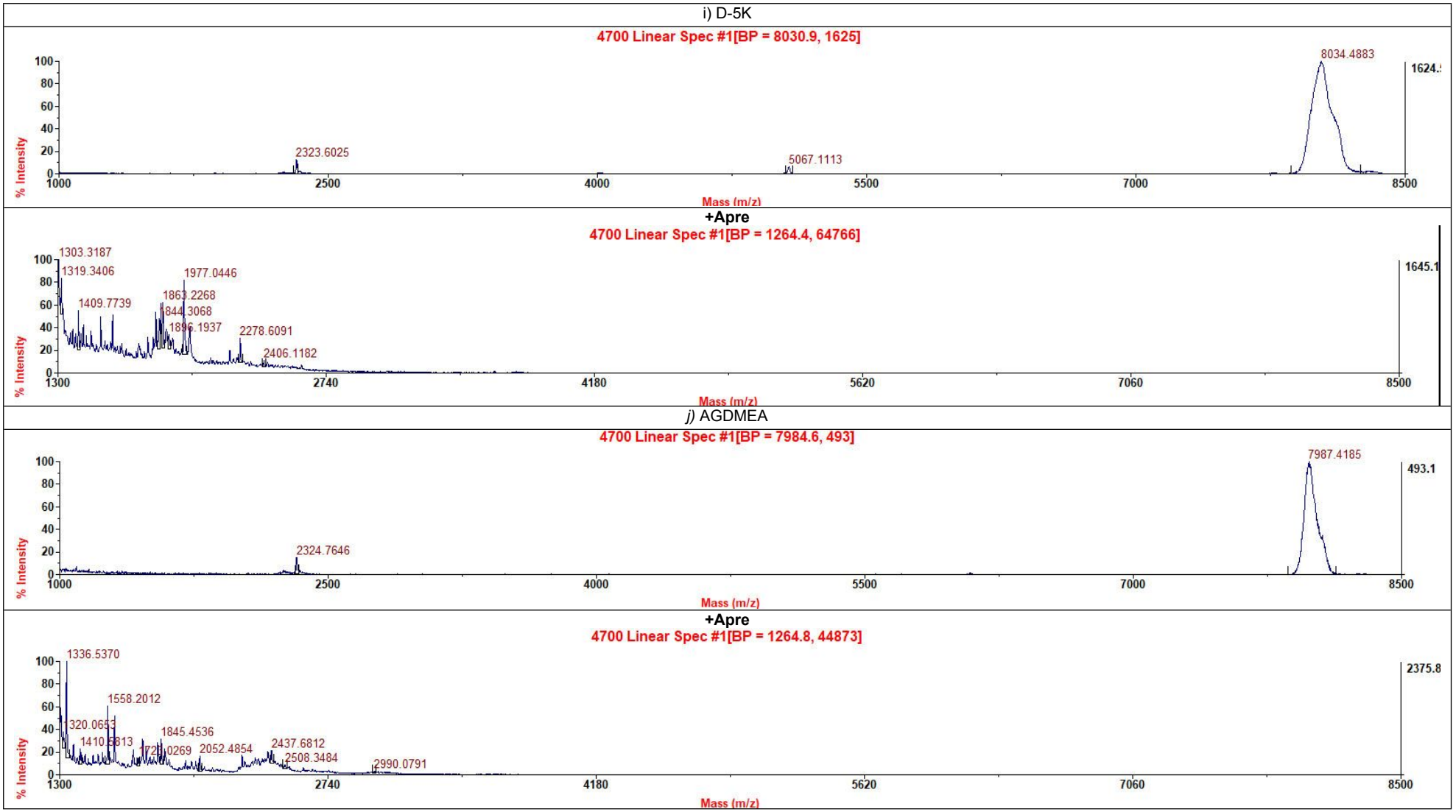
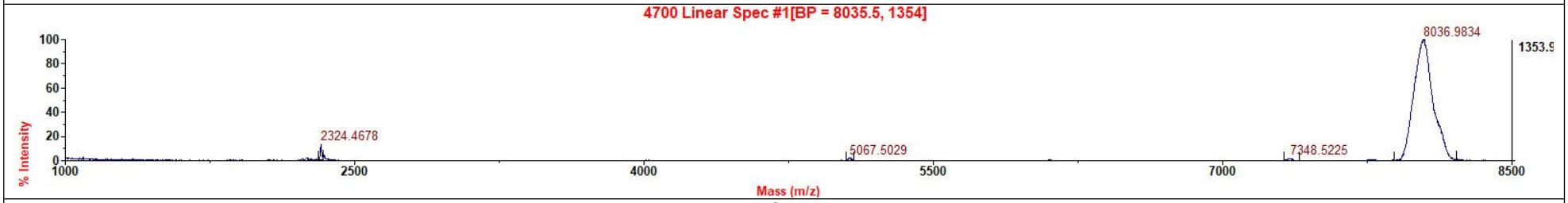



i) D-5K
4700 Linear Spec #1[BP = 8030.9, 1625]
8034.4883
2323.6025
5067.1113
% Intensity
Mass (m/z)
+Apre
4700 Linear Spec #1[BP = 1264.4, 64766]
1303.3187
1319.3406
1977.0446
1863.2268
1409.7739
1844.3068
1896.1937
2278.6091
2406.1182
1645.1
j) AGDMEA
4700 Linear Spec #1[BP = 7984.6, 493]
7987.4185
493.1
2324.7646
+Apre
4700 Linear Spec #1[BP = 1264.8, 44873]
1336.5370
1558.2012
1845.4536
2052.4854
2437.6812
2508.3484
2990.0791
2375.8

k) DMEAAA



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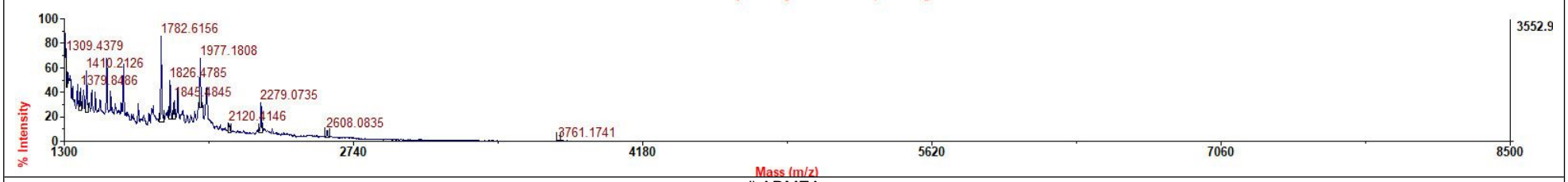
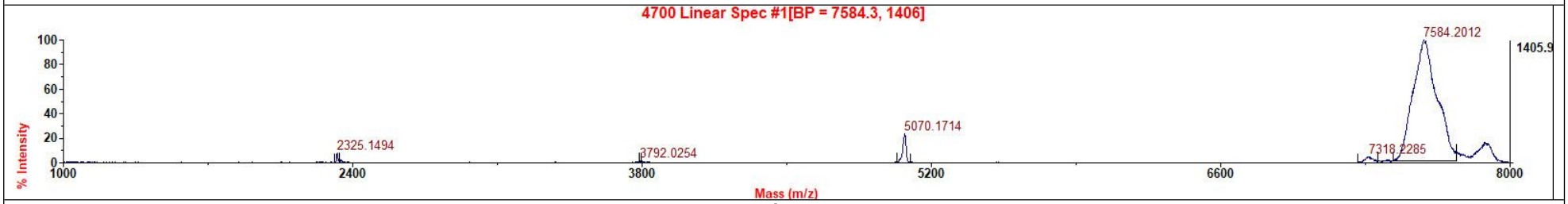


l) ΔDMEA



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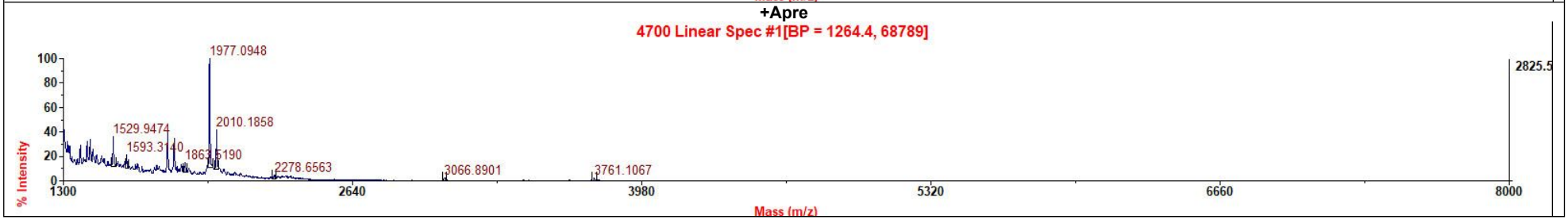
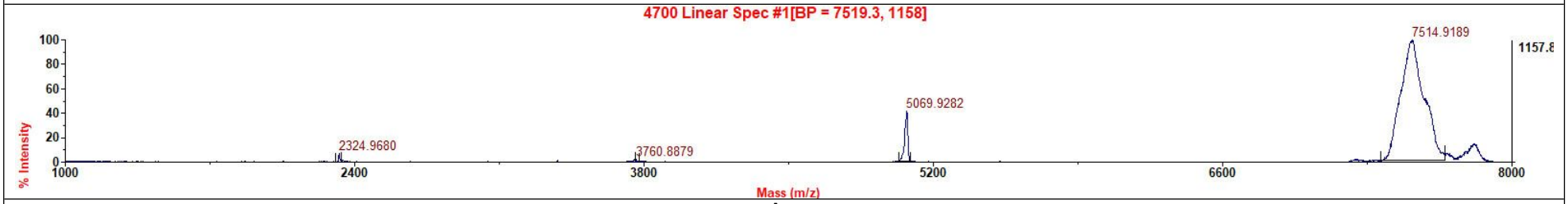


m) ΔDMEAA



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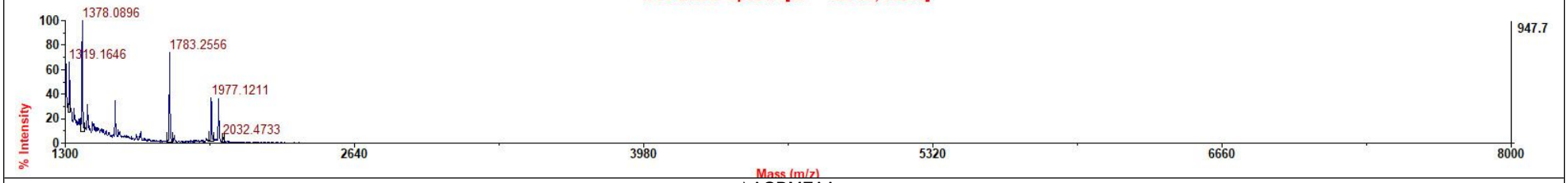
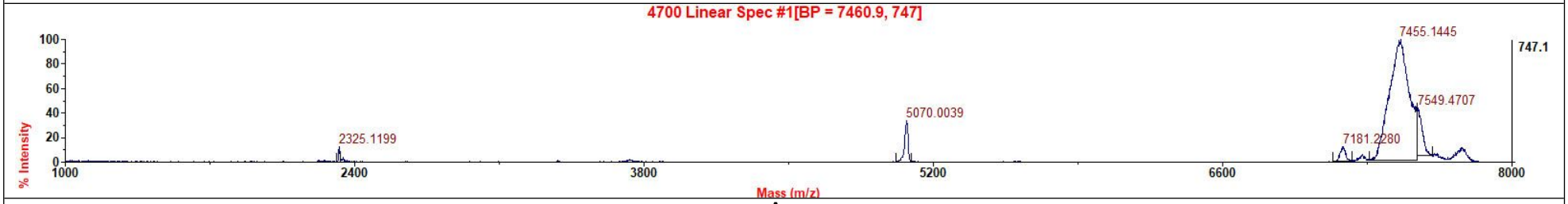


n) ΔGDMEAA



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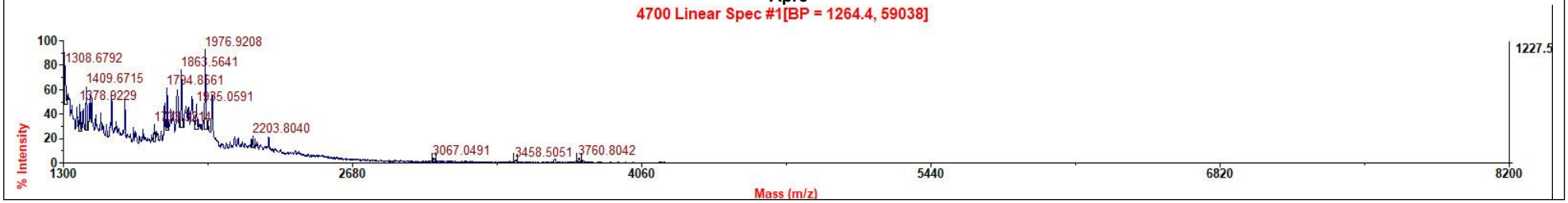
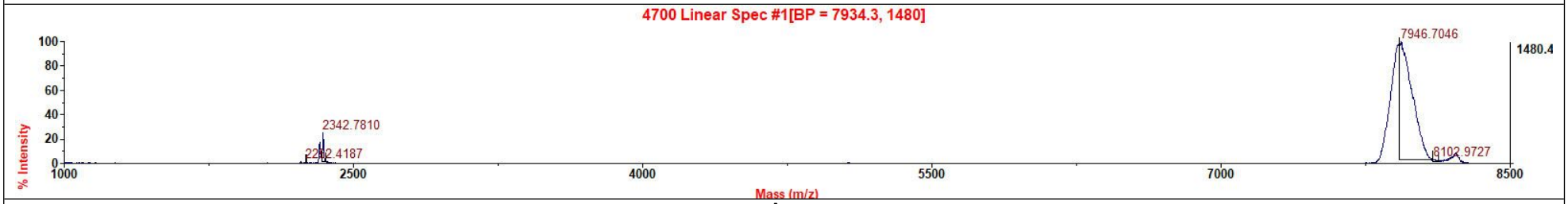


o) ΔA-1



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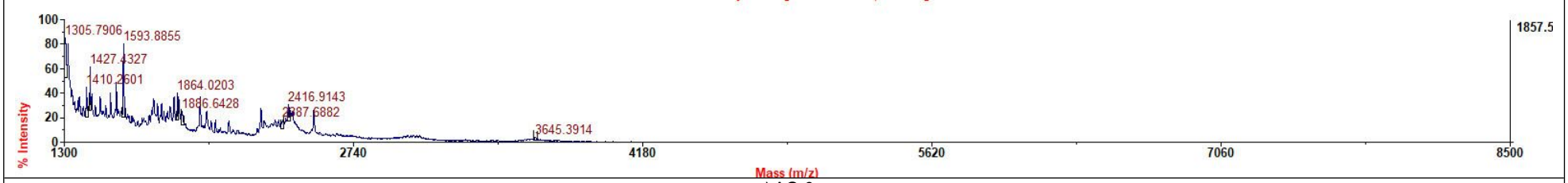
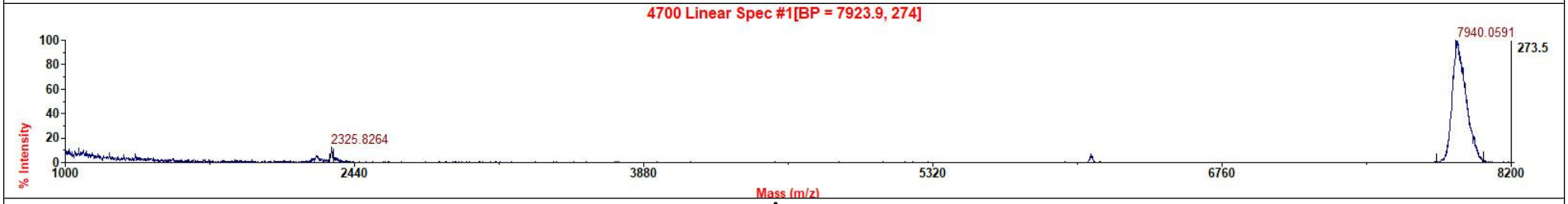


p) ΔG-6



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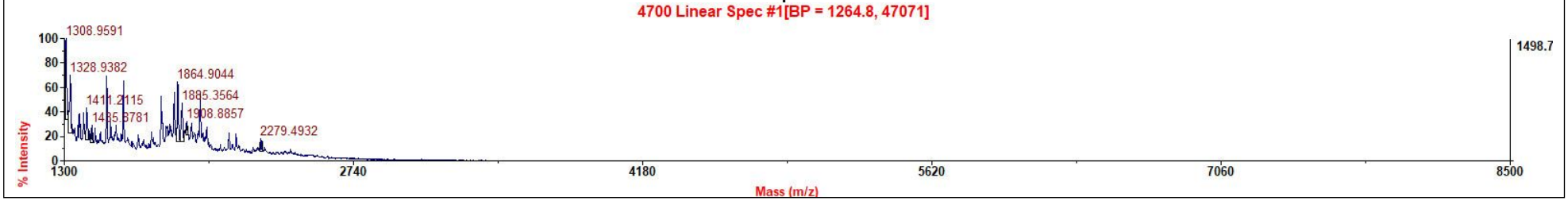
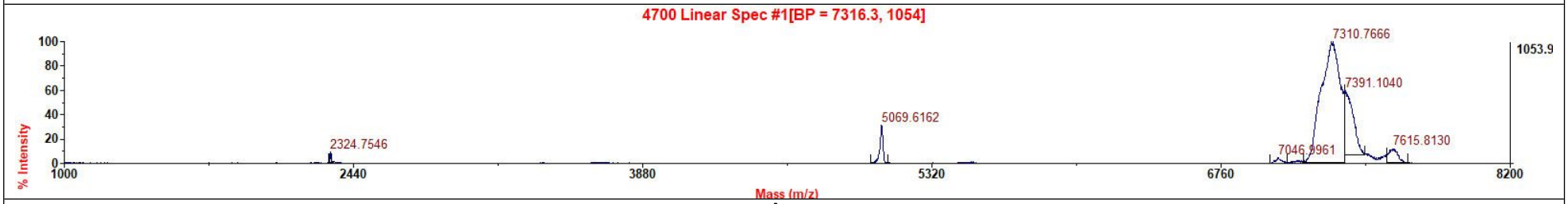


q) ΔK-11



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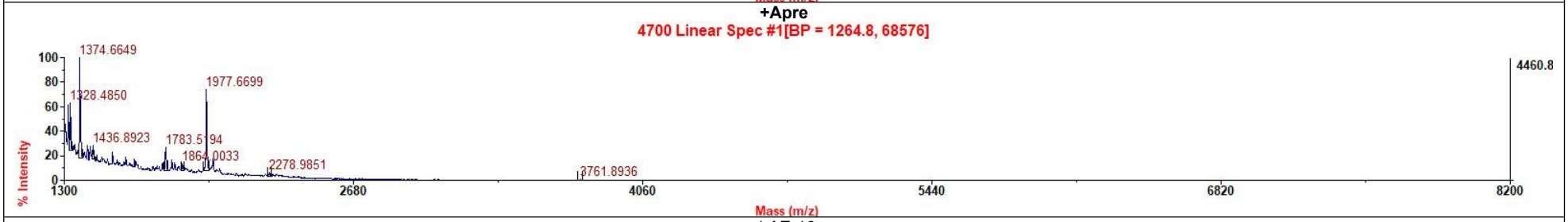
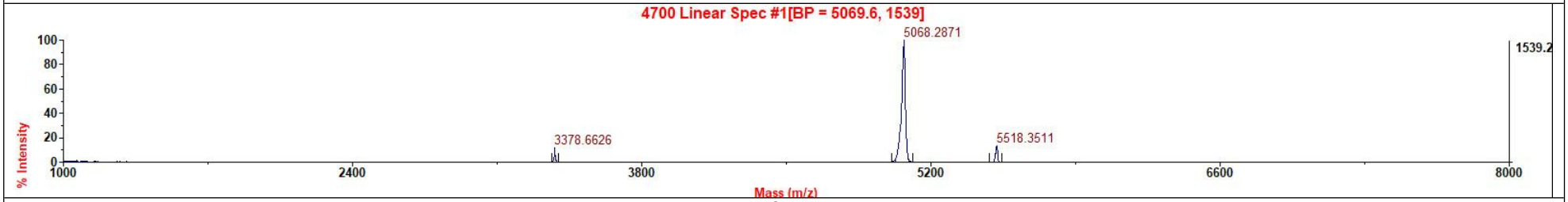


r) ΔE-16



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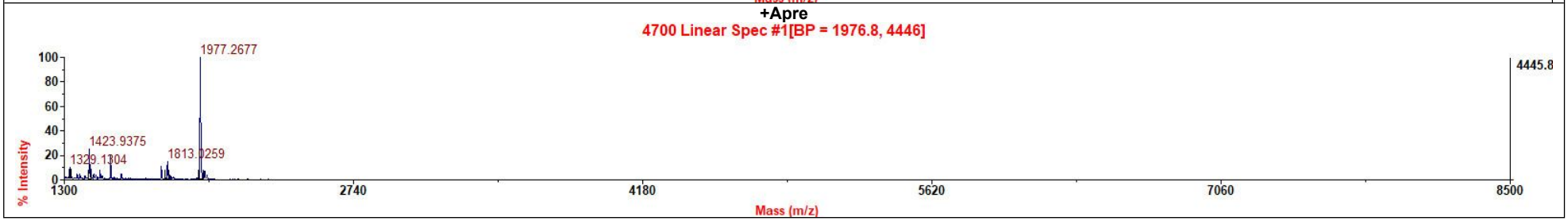
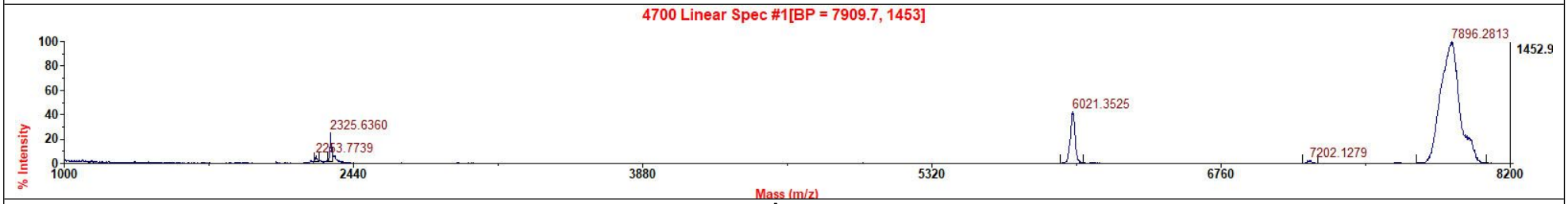


s) ΔF-21



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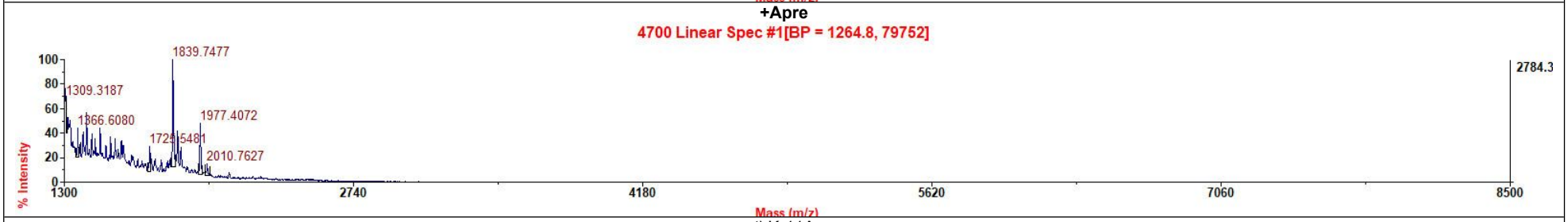
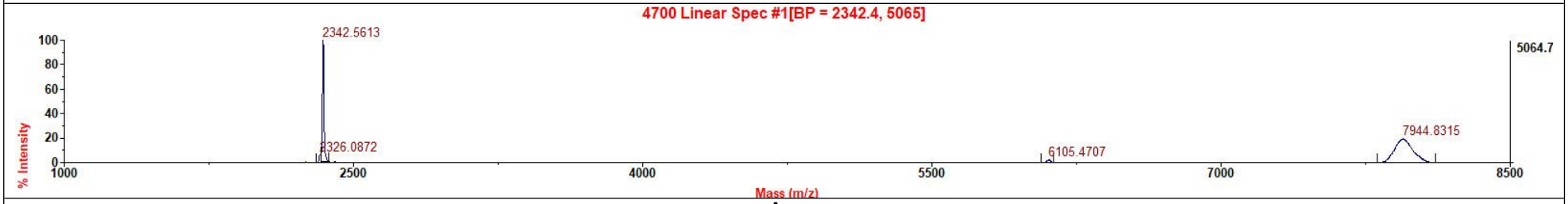


t) K-11A



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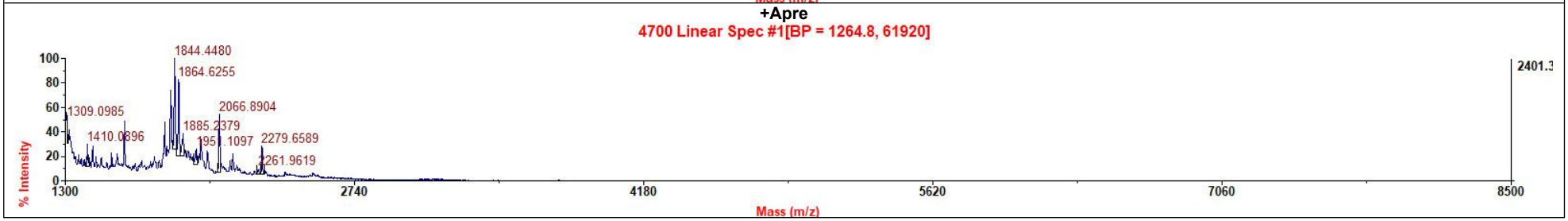
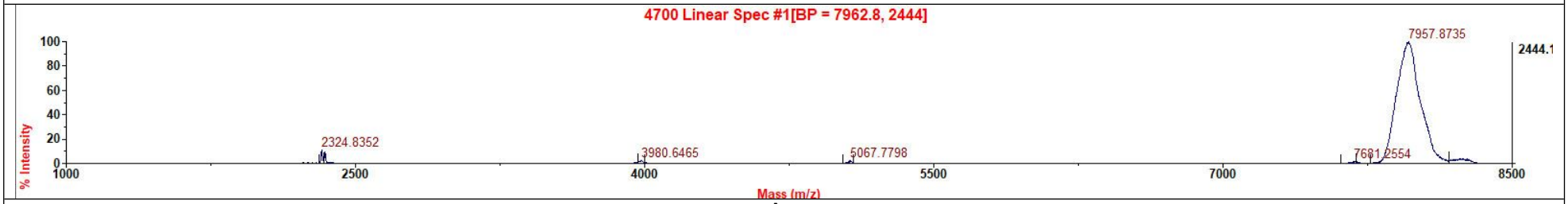


u) E-16A



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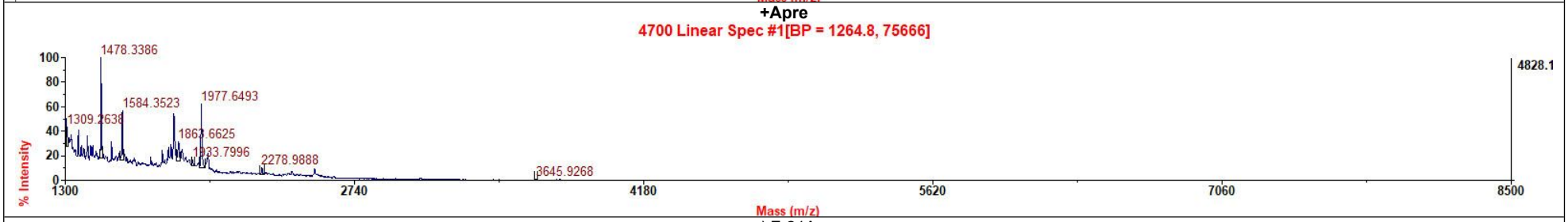
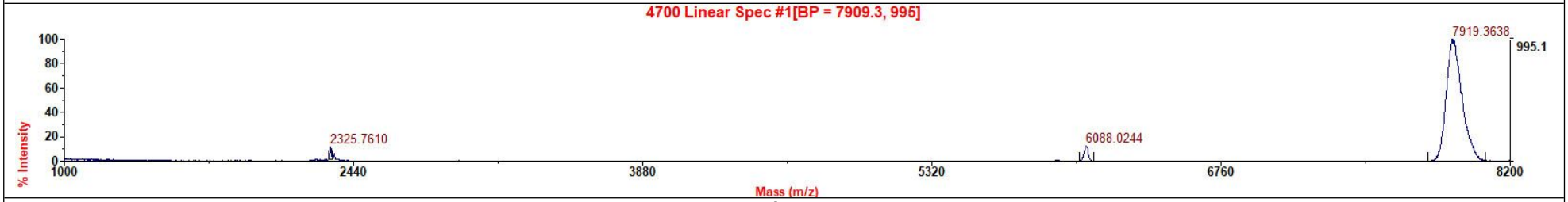


v) F-21A



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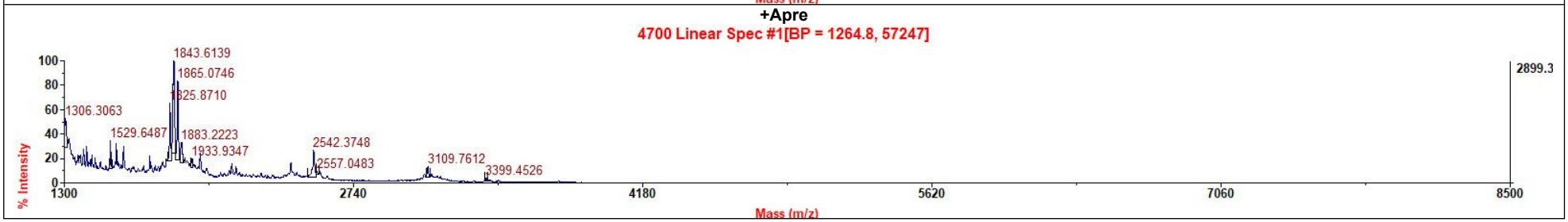
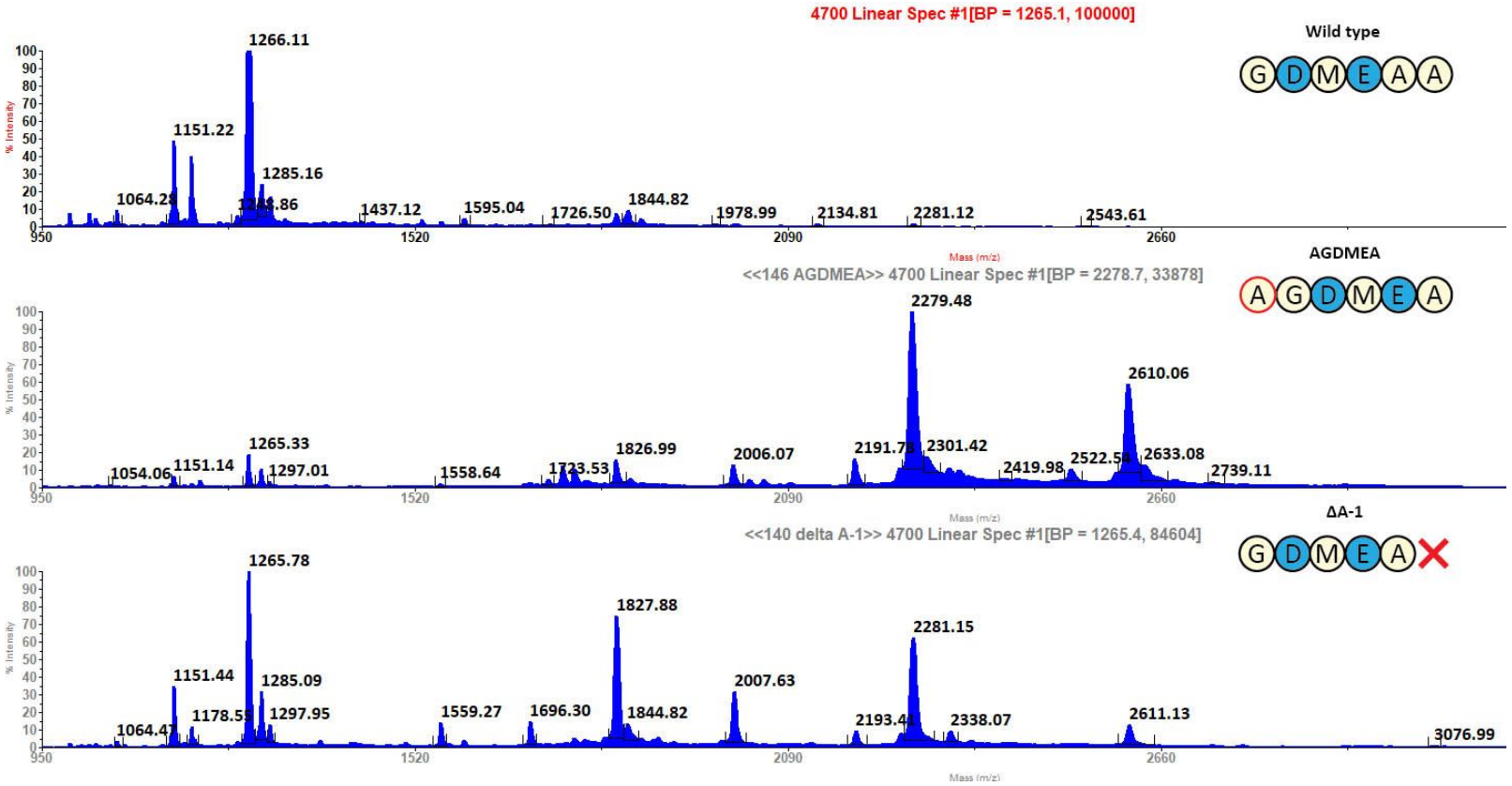


S6. Comparison of AprE digestion patterns wild type, AGDMEA and ΔA-1

If the lack of activity detected for the AGDMEA and ΔA-1 mutants was caused by AprE no longer being able to cleave the mersacidin leader, peaks larger than the mersacidin mass (1826 Da) should be visible by MALDI-TOF analysis. While the detected peaks that have a higher mass than mersacidin, have a much higher intensity than those in the wild type, these peaks are fragments of the leader peptide not containing mersacidin. Of both tested mutants, the fragments containing mersacidin and up to five amino acids from the leader peptide, should differ one alanine residue in mass. These differences are not detected. In fact, Masses resembling the peak of mersacidin are detected in both the AGDMEAA and ΔA-1 mutant's spectra. This shows that AprE can still cleave before the core peptide sequence. The fact that no activity is detected from these cleaved peptides confirms the results from the free cysteine essay, that not all rings have been formed.



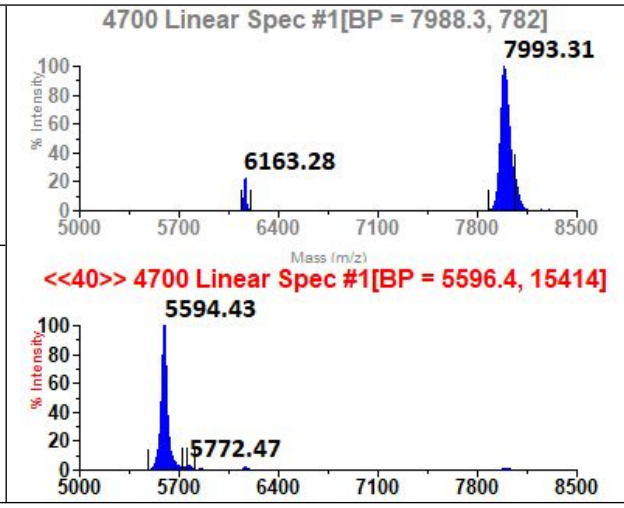
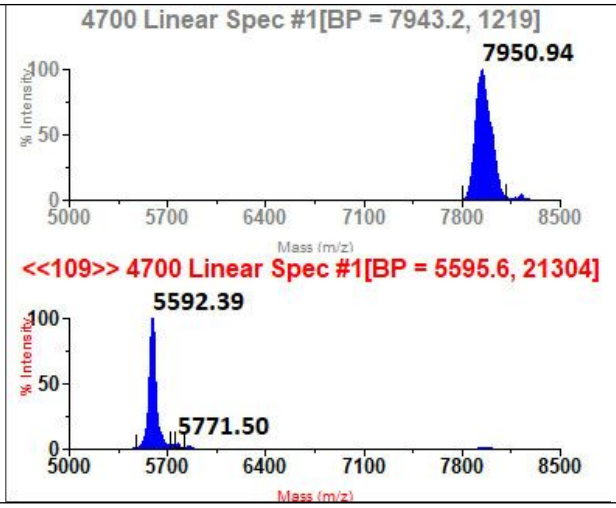
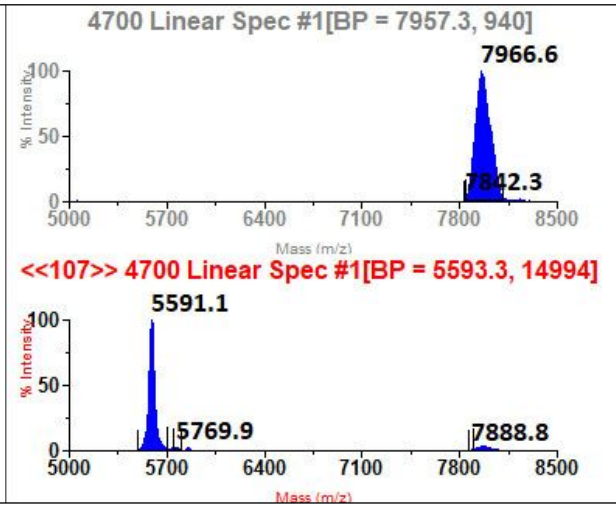
S7. Table of theoretical average masses

Theoretical average masses of all leader mutants in Dalton

		Unmodified	-CO_2	-CO_2 -1 H_2O	-CO_2 -2 H_2O	-CO_2 -3 H_2O	-CO_2 -4 H_2O	-CO_2 -5 H_2O
a	**Wild type (His6-MrsA)**	8108,99	8062,99	8044,97	8026,96	8008,94	7990,93	7972,91
b	pACYC E-3A + MrsM	8050,96	8004,95	7986,94	7968,92	7950,91	7932,89	7914,88
c	pACYC D-5A + MrsM	8064,98	8018,98	8000,96	7982,95	7964,93	7946,92	7928,90
d	pACYC E-3Q + MrsM	8108,01	8062,00	8043,99	8025,97	8007,96	7989,94	7971,93
e	pACYC D-5N + MrsM	8108,01	8062,00	8043,99	8025,97	8007,96	7989,94	7971,93
f	pACYC E-3A D-5A + MrsM	8006,95	7960,94	7942,93	7924,91	7906,90	7888,88	7870,87
g	pACYC E-3Q N-5A + MrsM	8107,02	8061,02	8043,00	8024,99	8006,97	7988,96	7970,94
h	pACYC E-3K + MrsM	8108,05	8062,05	8044,03	8026,01	8008,00	7989,98	7971,97
i	pACYC D-5K + MrsM	8122,08	8076,07	8058,06	8040,04	8022,03	8004,01	7986,00
j	pACYC AGDMEA + MrsM	8108,99	8062,99	8044,97	8026,96	8008,94	7990,93	7972,91
k	pACYC DMEAAA + MrsM	8123,02	8077,01	8059,00	8040,98	8022,97	8004,95	7986,94
l	pACYC ΔDMEA + MrsM	7662,52	7616,51	7598,50	7580,48	7562,46	7544,45	7526,43
m	pACYC ΔDMEAA + MrsM	7591,44	7545,43	7527,42	7509,40	7491,39	7473,37	7455,36
n	pACYC ΔGDMEAA + MrsM	7534,39	7488,38	7470,37	7452,35	7434,33	7416,32	7398,30
o	pACYC ΔA-1 + MrsM	8037,92	7991,91	7973,89	7955,88	7937,86	7919,85	7901,83
p	pACYC ΔG-6 + MrsM	8051,94	8005,94	7987,92	7969,90	7951,89	7933,87	7915,86
q	pACYC ΔK-11 + MrsM	7980,82	7934,81	7916,80	7898,78	7880,77	7862,75	7844,74
r	pACYC ΔE-16 + MrsM	7979,88	7933,87	7915,86	7897,84	7879,83	7861,81	7843,80
s	pACYC ΔF-21 + MrsM	7961,82	7915,81	7897,80	7879,78	7861,77	7843,75	7825,74
t	pACYC K-11A + MrsM	8051,90	8005,89	7987,88	7969,86	7951,85	7933,83	7915,82
u	pACYC E-16A + MrsM	8050,96	8004,95	7986,94	7968,92	7950,91	7932,89	7914,88
v	pACYC F-21A + MrsM	8032,90	7986,89	7968,88	7950,86	7932,85	7914,83	7896,82
Mersacidin leader +GDMEAA		6164,71						
Mersacidin leader -GDMEAA		5590,10						

S8. MrsT150-His cleavage analysis

The peptides, as verified by LC-MS mass analysis, were incubated with MrsT150-His, and analyzed by MALDI-TOF analysis. The large size prevents accurate depiction of the masses of individual dehydration states, but still gives a precise centroid mass. The mersacidin leader -GDMEAA theoretical average mass is 5590,10 Da (with the exception of variants where residues upstream of GDMEAA were mutated), which can be detected if MrsT150-His can process the leader mutant.

<table>
<tr><td colspan="3">a) Wild type</td></tr>
<tr><td>No MrsT</td><td rowspan="2"> 
</td><td rowspan="2">Is processed by MrsT150-His</td></tr>
<tr><td>+MrsT150-His</td></tr>
<tr><td colspan="3">b) E-3A</td></tr>
<tr><td>No MrsT</td><td rowspan="2"> 
</td><td rowspan="2">Is processed by MrsT150-His</td></tr>
<tr><td>+MrsT150-His</td></tr>
<tr><td colspan="3">c) D-5A</td></tr>
<tr><td>No MrsT</td><td rowspan="2"> 
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<tr><td>+MrsT150-His</td></tr>
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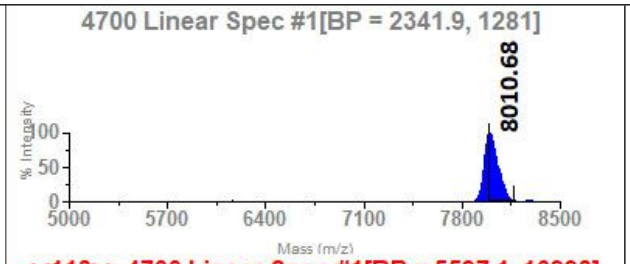
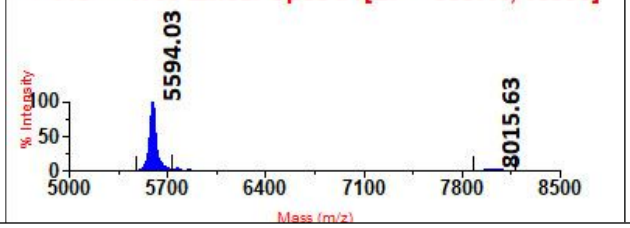
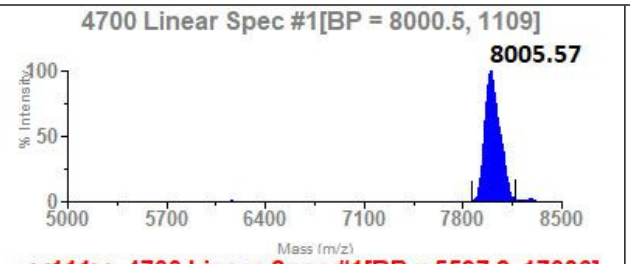
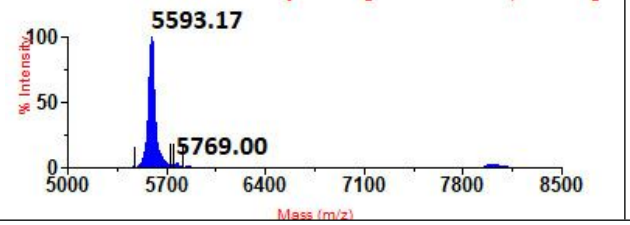
d) D-5N		
No MrsT		Is processed by MrsT150-His
+MrsT150-His		
e) E-3Q		
No MrsT		Is processed by MrsT150-His
+MrsT150-His		
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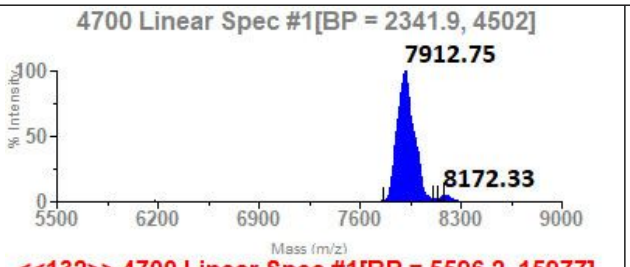
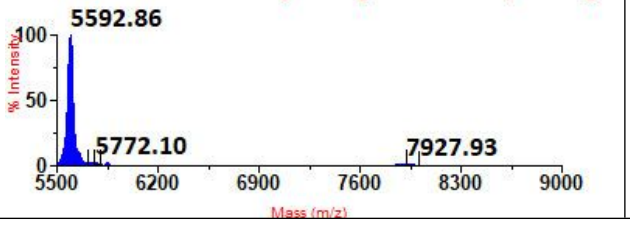
No MrsT		Is processed by MrsT150-His
+MrsT150-His		

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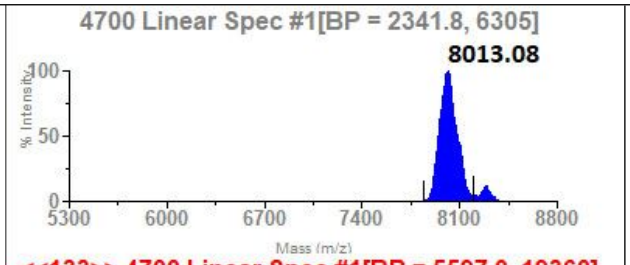
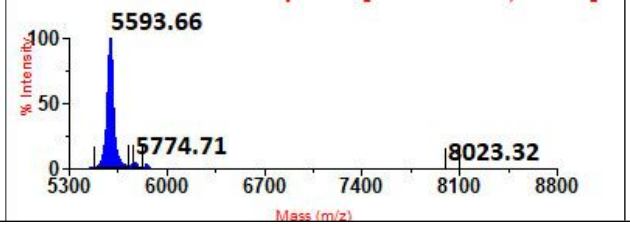
No MrsT		Is processed by MrsT150-His
+MrsT150-His		
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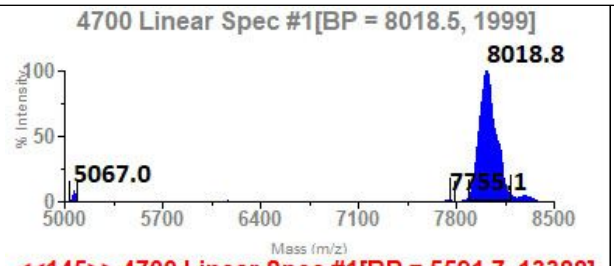
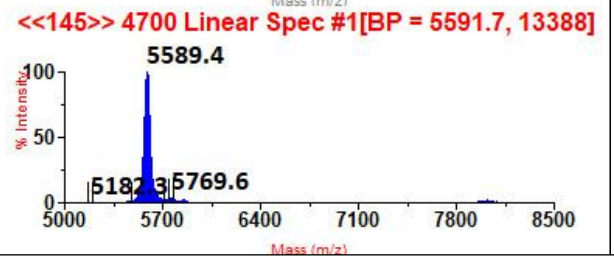
No MrsT		Is processed by MrsT150-His
+MrsT150-His		
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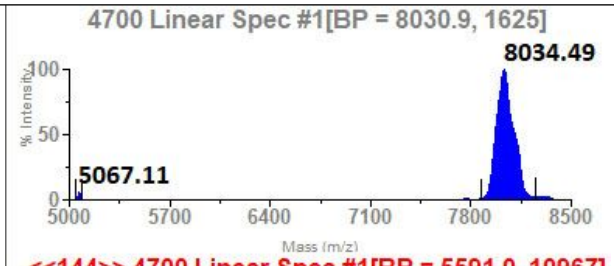
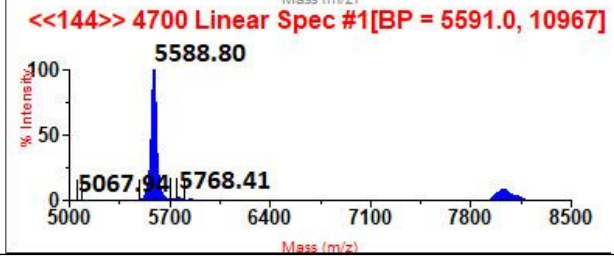
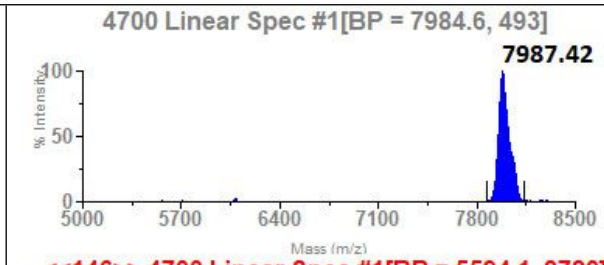
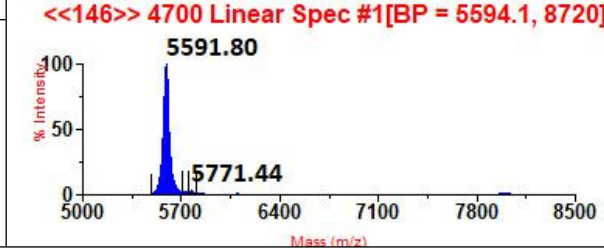
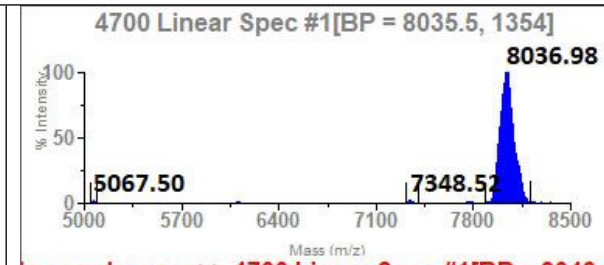
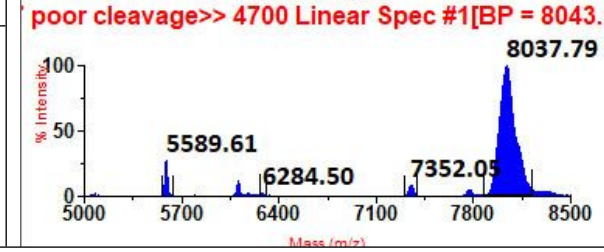
No MrsT		Is processed by MrsT150-His
+MrsT150-His		

j) AGDMEA		
No MrsT		Is processed by MrsT150-His
+MrsT150-His		
k) DMEAAA		
No MrsT		Is not processed well by MrsT150-His
+MrsT150-His		
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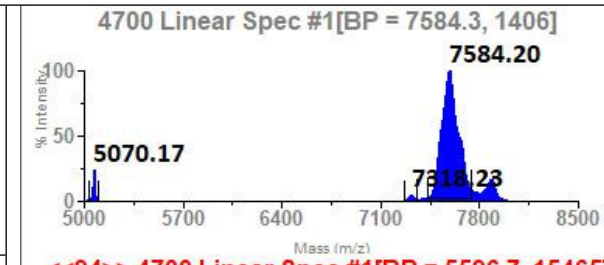
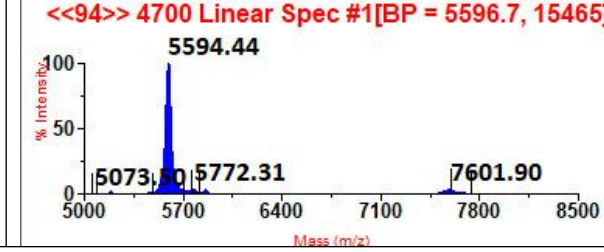
No MrsT		Is processed by MrsT150-His
+MrsT150-His		

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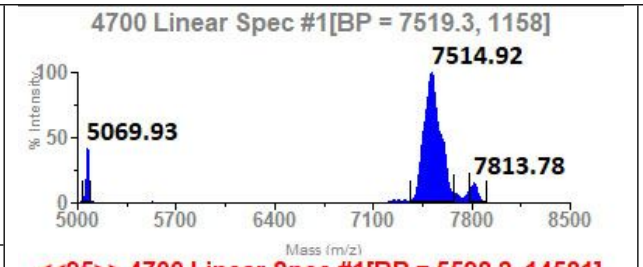
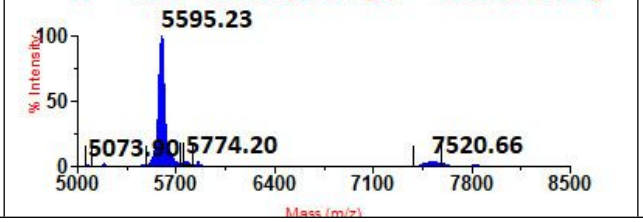
No MrsT		Is processed by MrsT150-His
+MrsT150-His		
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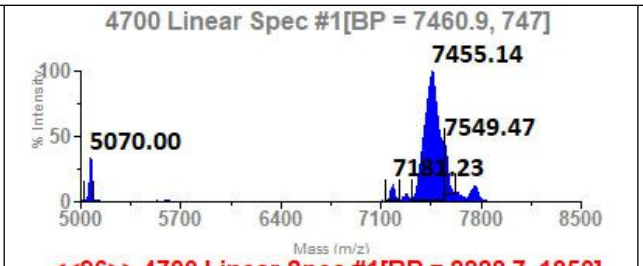
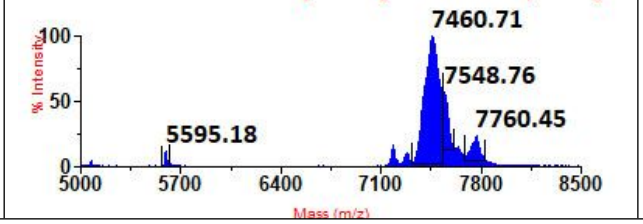
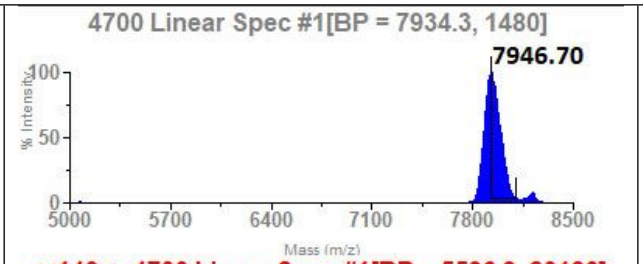
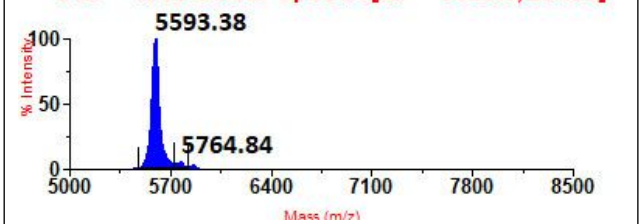
No MrsT		Is not processed by MrsT150-His
+MrsT150-His		
o) ΔA-1		
No MrsT		Is processed by MrsT150-His
+MrsT150-His		

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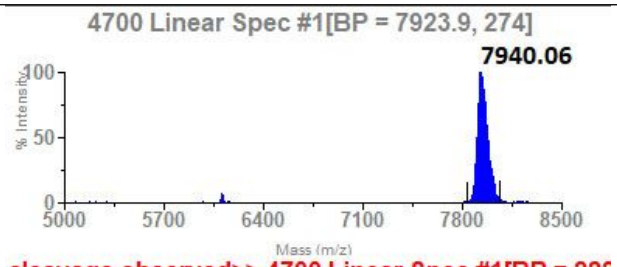
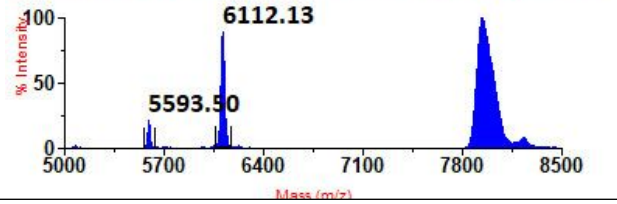
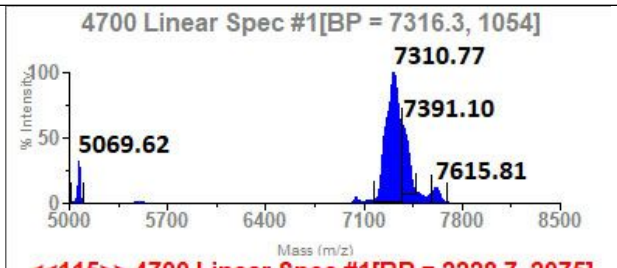
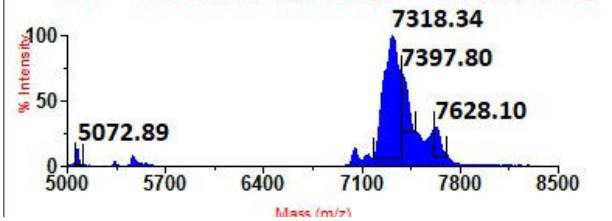
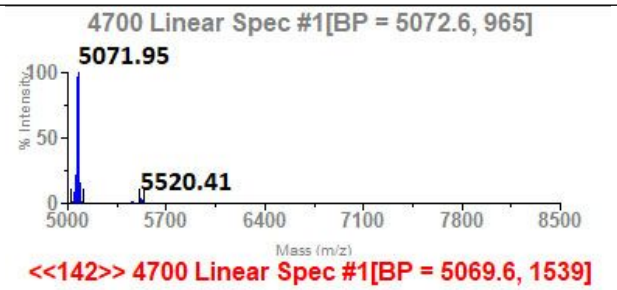
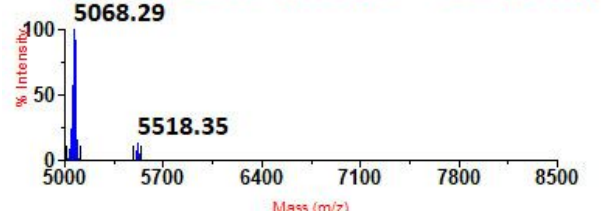
No MrsT		Is not processed by MrsT150-His
+MrsT150-His	cleavage observed>> 4700 Linear Spec #1[BP = 222 	
q) ΔK-11		
No MrsT		Is not processed by MrsT150-His
+MrsT150-His	<<115>> 4700 Linear Spec #1[BP = 2228.7, 2075] 	
r) ΔE-16		
No MrsT		Degraded prior to MrsT150-His digestion
+MrsT150-His	<<142>> 4700 Linear Spec #1[BP = 5069.6, 1539] 	

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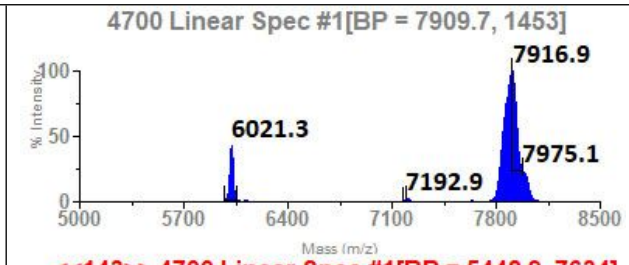
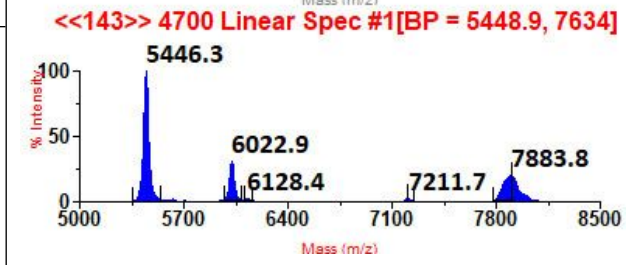
<tr><td>No MrsT</td><td rowspan="2"> 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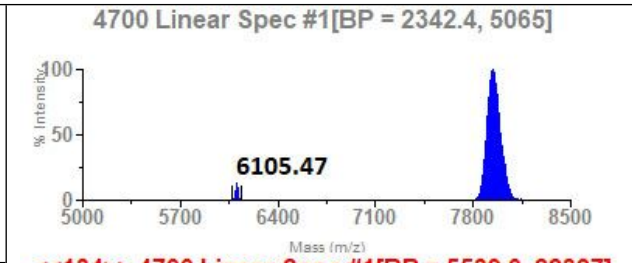
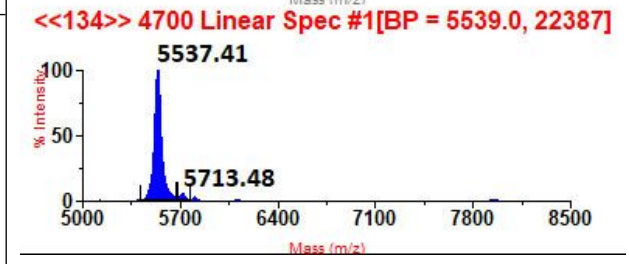
<tr><td>No MrsT</td><td rowspan="2"> 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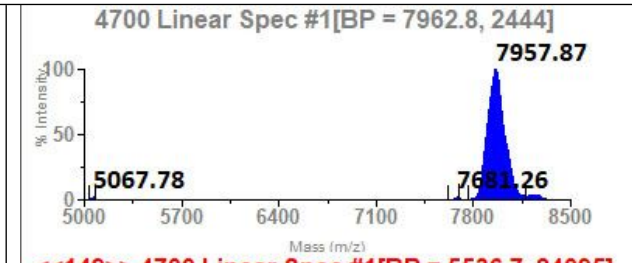
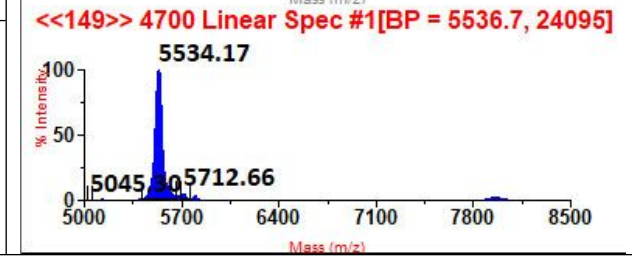
<tr><td>No MrsT</td><td rowspan="2"> 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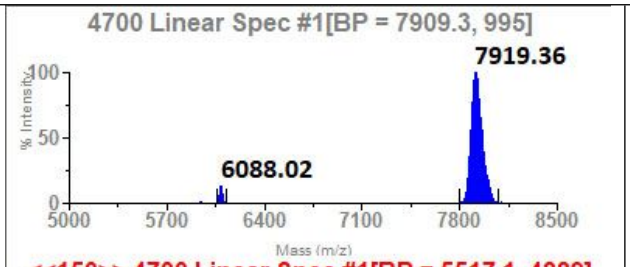
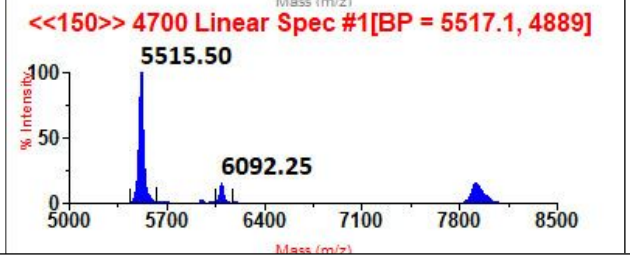
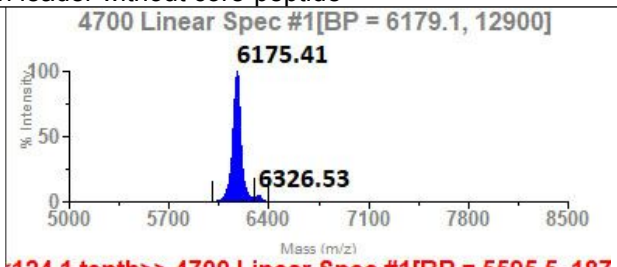
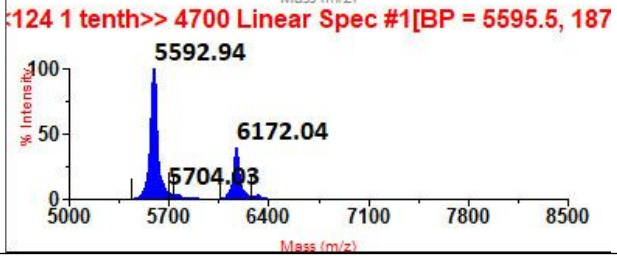
v) F-21A		
No MrsT		Is processed by MrsT150-His
+MrsT150-His		
His6-mersacidin leader without core-peptide		
No MrsT		Is processed by MrsT150-His
+MrsT150-His		

S9. List of primers used in this study

	Sequence	Template	Result
fw	GACGGGTCTCCGGCGGCAGCATGTACTTTTACATTGCCTGGTG	pACYC His-MrsA + MrsM[1]	pACYC E-3A + MrsM
rv	GACGGGTCTCCCGCCATGTCTCCCGCACCTACTAACTTATCC		
fw	GACGGGTCTCCAGCGATGGAAGCAGCATGTACTTTTACATTG		pACYC D-5A + MrsM
rv	GACGGGTCTCCCGCTCCCGCACCTACTAACTTATCCATTTG		
fw	GACGGGTCTCCGCAGGCAGCATGTACTTTTACATTGCCTGGTG		pACYC E-3Q + MrsM
rv	GACGGGTCTCCCTGCATGTCTCCCGCACCTACTAACTTATCC		
fw	GACGGGTCTCCAAACATGGAAGCAGCATGTACTTTTACATTG		pACYC D-5N + MrsM
rv	GACGGGTCTCCGTTTCCCGCACCTACTAACTTATCCATTTG		
fw	GACGGGTCTCCATGGCGGCAGCATGTACTTTTACATTGCCTGGTG		pACYC E-3A
rv	GACGGGTCTCCCCATCGCTCCCGCACCTACTAACTTATCCATTTGTG		D-5A + MrsM
fw	GACGGGTCTCCATGCAGGCAGCATGTACTTTTACATTGCCTGGTG		pACYC E-3Q
rv	GACGGGTCTCCGCATGTTTCCCGCACCTACTAACTTATCCATTTGTG		N-5A + MrsM
fw	GACGGGTCTCCGAAAGCAGCATGTACTTTTACATTGCCTGGTGG		pACYC E-3K
rv	GACGGGTCTCCTTTCATGTCTCCCGCACCTACTAACTTATCC		+ MrsM
fw	GACGGGTCTCCAAAGATGGAAGCAGCATGTACTTTTACATTGCCTG		pACYC D-5K
rv	GACGGGTCTCCCTTTCCCGCACCTACTAACTTATCCATTTGTGC		+ MrsM
fw	GACGGGTCTCCAGACATGGAAGCATGTACTTTTACATTGCCTGGTGGCGG		pACYC
rv	GACGGGTCTCCGTCTCCCGCTGCACCTACTAACTTATCCATTTGTGCTTCTTTCAGCTC		AGDMEA + MrsM
fw	GACGGGTCTCCAAGCAGCAGCGTGTACTTTTACATTGCCTGGTGGCGG		pACYC
rv	GACGGGTCTCCGCTTCCATGTCCGCACCTACTAACTTATCCATTTGTGCTTCTTTTC		DMEAAA + MrsM
fw	GACGGGTCTCGGAGCATGTACTTTTACATTGCCTGG		pACYC
rv	GACGGGTCTCGGCTCCCGCACCTACTAACTTATCC		ΔDMEA + MrsM
fw	GACGGGTCTCGGAGCATGTACTTTTACATTGCCTGG		pACYC
rv	GACGGGTCTCGGATGTACTTTTACATTGCCTGGTGG		ΔDMEAA + MrsM
fw	GACGGGTCTCGGAGCATGTACTTTTACATTGCCTGG		pACYC
rv	GACGGGTCTCGCATCCCGCACCTACTAACTTATCC		ΔGDMEAA + MrsM
fw	GACGGGTCTCCCATGTACTTTTACATTGCCTGGTGGCGG		pACYC ΔA-1
rv	GACGGGTCTCCCATGCTTCCATGTCTCCCGCACCTAC		+ MrsM
fw	GACGGGTCTCCCGGACATGGAAGCAGCATGTACTTTTACATTGCC		pACYC ΔG-6
rv	GACGGGTCTCCTCCGCACCTACTAACTTATCCATTTGTGCTTCTTTC		+ MrsM
fw	GACGGGTCTCCTAGGTGCGTGTACTTTTACATTGCCTGGTGGCG		pACYC ΔK-11
rv	GACGGGTCTCCCCTACTAAATCCATTTGTGCTTCTTTCAGCTCACTGAATG		+ MrsM
fw	GACGGGTCTCCAAGCACAAATGGATAAGTTAGTAGGTGCGGGAG		pACYC ΔE-16
rv	GACGGGTCTCCGCTTTCAGCTCACTGAATGGGTTACCAGCTG		+ MrsM
fw	GACGGGTCTCCCAAGTGAGCTGAAAGAAGCACAAATGGATAAGTTAGTAG		pACYC ΔF-21
rv	GACGGGTCTCCCTTGGGTTACCAGCTGGATTTTGTGTAGAATTTTC		+ MrsM
fw	GACGGGTCTCCGCGTTAGTAGGTGCGGGAGACATGGAAG		pACYC K-11A
rv	GACGGGTCTCCACGCATCCATTTGTGCTTCTTTCAGCTCACTGAA		+ MrsM
fw	GACGGGTCTCCAGCGGCACAAATGGATAAGTTAGTAGGTGCGGGAGAC		pACYC E-16A
rv	GACGGGTCTCCCGCTTTCAGCTCACTGAATGGGTTACCAGCTG		+ MrsM
fw	GACGGGTCTCCAGCGAGTGAGCTGAAAGAAGCACAAATGGATAAGTTAG		pACYC F-21A
rv	GACGGGTCTCCCGCTGGGTTACCAGCTGGATTTTGTGTAGAATTTTC		+ MrsM

References

(1) Viel, J. H.; Jaarsma, A. H.; Kuipers, O. P. Heterologous Expression of Mersacidin in Escherichia Coli Elucidates the Mode of Leader Processing. *ACS Synth. Biol.* **2021**, *10* (3), 600–608. https://doi.org/10.1021/acssynbio.0c00601.