Identifier		Solvent	Type of sample preparation and/or sample introduction 1	2
13579A		5% FA	None	
72079		0.1% FA then 50/50 ACN/water + 0.	ON-HPLC	ON-HPLC
715		0.1%TFA/50%ACN	ON-HPLC	ON-HPLC
20107	V	50% MeOH in aq. FA (0.1%)	SN	SN
26019		5% FA/60% ACN	SN	SN
65214		H2O:ACN 1:1, 0.1% TFA	None	OFF-HPLC
10266	V	5% aqueous FA	ON-HPLC	ON-HPLC
46011		0.1 % TFA/50% ACN		
dsu	V	0.1 % TFA 50 % ACN	DD HCCA	DDHCCA
12800		0.1%TFA in 50%ACN	micropurification after derivatisation	micropurification after derivatisa
78364		5% FA	SN/OM-HPLC	SN/OM-HPLC
13579B	V	50%ACN +0.1%TFA	ON-HPLC	ON-HPLC
55000		ACN/H2O	None/ON-HPLC	
51565		0.1% TFA / 50% ACN	None	
30109		50% ACN 0.1% TFA	None	
11010		50% ACN/0.1% TFA	OM-HPLC/ON-HPLC	OM-HPLC/ON-HPLC
47223		50% ACN, 0.1%TFA	cystallization with CHCAon a MALDI target pl	cystallization with CHCA on a MALD
04318	V	0.1% FA/ACN (50:50)	DD	DD
51952		5%FA/ 2% ACN	ON-HPLC	
99999		5% ACN 0.2% FA		ON-HPLC
73108		50% ACN+0.1%TFA	ON-HPLC	ON-HPLC
17999		5 % FA	ON-HPLC	ON-HPLC
98166		5% FA	ON-HPLC	ON-HPLC
27406		5% FA	ON-HPLC	ON-HPLC
91741		20 uL 0.1% TFA:ACN	spotted sample in 5 mg/mL CHCA matrix (1:1 0	see peptide 1 for all up to questi
91573		5 % FA	SN	SN/ON-HPLC
51583	V	50% ACN, 0.1%TFA	MALDI	MALDI
70091		50% ACN/ 0.1% TFA	OM-HPLC	OM-HPLC
19351		5 % FA	SN	SN
27974		50% ACN, 1% FA	SN/OM-HPLC	SN
17017		50:50 ACN:water + 0.1% TFA	OFF-HPLC/OM-HPLC	OFF-HPLC/OM-HPLC
12144		water, ACN, TFA	ON-HPLC	ON-HPLC
32466		5% FA, 10 min sonication, dissol	SN	SN/MALDI
78544		5 % FA	ON-HPLC	ON-HPLC
1467		5 % FA	OM-HPLC	ON-HPLC
52104		5% FA	SN/OMALDI	
80053		5 % FA	SPE	
54321		0.02%TFA in H2O	ON-HPLC	ON-HPLC
85035	V	0.1% FA in Water	ON-HPLC	ON-HPLC
10523	V	0.1% TFA		
87458		50%ACN/0.1%TFA		
1605		50%ACN, 0.1%FA	SN	SN
12345		0.1%TFA in 50% ACN	None	
11747		10:1 0.1M AA:ACN	ON-HPLC	ON-HPLC
7974		50% ACN/H2O with 0.1%TFA	ON-HPLC	
49495		water	None	
47551		5 microL ACN 70%.1%TFA+ 55microL	OFF-HPLC	
11089		5 % FA		

			Type of Instrument	
3	**4**	**5**	**1**	**2**
			MS	MS
ON-HPLC		SN	MS	MS
ON-HPLC	ON-HPLC	ON-HPLC	Edman/MS	Edman/MS
SN	SN	SN	MS	MS
SN	SN	SN	MS	MS
			MS	MS
ON-HPLC	ON-HPLC	ON-HPLC	MS	MS
			MS	MS
DDHCCA	DDHCCA	DD	MS	MS
micropurification after derivatisa	micropurification after derivatisation		MS	MS
SN/OM-HPLC	SN/OM-HPLC	SN/OM-HPLC	MS	MS
	ON-HPLC	ON-HPLC	MS	MS
	ON-HPLC	ON-HPLC	MS	MS
			MS	MS
			MS	MS
OM-HPLC/ON-HPLC	OM-HPLC/ON-HPLC	OM-HPLC/ON-HPLC	Edman/MS	Edman/MS
cystallization with CHCA on a MALD	cystallization with CHCA on a MALD	cystallization with CHCA on a MALDI	MS	MS
DD	DD	DD	MS	MS
ON-HPLC	ON-HPLC	ON-HPLC	MS	MS
ON-HPLC	ON-HPLC	ON-HPLC	MS	MS
ON-HPLC	ON-HPLC	ON-HPLC	MS	MS
	ON-HPLC	ON-HPLC	MS	MS
ON-HPLC	ON-HPLC	ON-HPLC	MS	MS
	ON-HPLC	ON-HPLC	MS	MS
same answers as peptide 1 down to	same answers down to question 91	same answers as peptide 1 down to qu	MS	
SN/ON-HPLC	SN/OM-HPLC	SN/ON-HPLC	MS	MS
MALDI	MALDI	MALDI	MS	MS
	OM-HPLC	OM-HPLC	Edman/MS	Edman/MS
SN	SN		Edman/MS	Edman/MS
SN	SN	SN	MS	MS
OFF-HPLC/OM-HPLC	OFF-HPLC/OM-HPLC		MS	
ON-HPLC	ON-HPLC	ON-HPLC		
SN	SN	SN	MS	MS
	ON-HPLC		MS	MS
	ON-HPLC	ON-HPLC	MS	MS
SN	SN			MS
ON-HPLC	ON-HPLC	ON-HPLC		
	ON-HPLC	ON-HPLC	MS	
			MS	MS
			MS	MS
SN	SN	SN	MS	MS
			Edman/MS	Edman
ON-HPLC	ON-HPLC	ON-HPLC	MS	MS
			MS	
			MS	MS

cation			% of Peptide Consumed for AA					% coverage obtained by AA					% Peptide consumed by Edman sequencing (wi	
3	4	5	1	2	3	4	5	1	2	3	4	5	1	2
MS	MS	MS												
MS	MS	MS												
Edman/MS	Edman/MS	Edman/MS											90-100	80-90
MS	MS	MS												
MS	MS	MS												
MS	MS	MS												
MS	MS	MS												
MS	MS	MS												
MS	MS	MS						0						
MS	MS		10-2(	10-20				0						
MS	MS	MS												
MS	MS	MS												
MS	MS	MS												
MS	MS	MS	10-2(	10-2(	10-20			0						
MS	MS	MS			10-20			0						
Edman/MS	Edman/MS	Edman/MS											40% entire mix by	40% of mix was analyzed
MS	MS	MS												
MS	MS	MS												
MS	MS	MS						0						
MS	MS	MS												
MS	MS	MS	40-50			30-4(	30-40							
MS	MS	MS	20-3(	20-3(	20-30	20-3(	20-30							
MS	MS	MS												
MS	MS	MS						0						
MS	MS	MS												
MS	MS	MS						0			0			
Edman/MS	Edman/MS	Edman/MS											80-90	80-90
Edman/MS	Edman/MS													50 % of mixture used fo
MS	MS				10-20									
Edman/MS	MS					40-50								
	MS													
MS	MS	MS			20-30									
MS	MS		10-2(	10-20		10-20								
MS	MS	MS												
MS	MS													
MS	MS													
MS	MS													
MS	MS	MS												
Edman	Edman							0					90-100	90-100
MS	MS	MS						0						
MS	MS	MS				80-90								
													90-100	

.th prefractionation)			% of Peptide sequence obtained by Edman sequencing		
3	**4**	**5**	**1**	**2**	**3**
90-100	90-100	90-100	100		100
40% of mix was analyze	40% of mix was analyze	40% of mix was analyzed	83		
80-90	80-90	80-90	54		77
50 % of miture used fo	50 % of miture used for Edman			50 % in accordance with	100 % in accordance with
50-60					100
80-90	90-100		100	100	
			>90		

		% of Peptide consumed by MS (no prefractiona					% of Peptide sequence obtained by I					Type of M
4	5	1	2	3	4	5	1	2	3	4	5	1
		90-100	90-10(	90-100		90-100	100	100	100	100	100	MALDI
		30-40	30-40	30-40	10-20		100	100	100	100	100	ES
		<10	<10	<10	<10	<10	100	100	100	100	100	ES
		90-100	90-10(	90-100	90-100	90-100	100	100	100	100	100	ES
												ES
		<10	10-20	<10	<10	<10	100	100	100	100	100	MALDI
		<10	<10	<10	<10	<10	100	100	100	100	100	ES
							100	100	<50	100	100	MALDI
		<10		<10	<10	<10	100	100	100	100	100	MALDI
							100	100	100	100	100	MALDI
		90-100	90-10(	90-100	90-100	90-100	100	100	100	100	100	ES
												ES/MALDI
		20-30	10-20	10-20	10-20	10-20	100	100	100	100	100	ES/MALDI
		10-20	10-20	10-20	10-20	10-20	100	100	100	100	100	MALDI
		10-20	10-20	10-20	10-20	10-20	100	100	100	100	100	MALDI
79	10 residues	40-50	40-50	40-50	40-50	40-50		83	100	79	58	
		10-20	10-20	10-20	10-20	10-20	100	100	100	100	100	MALDI
							100	100		100	100	MALDI
		60-70	60-70	60-70	60-70	60-70	100	100	100	100	40	ES
		<10	<10	<10	<10	<10	100	100	100	100	50	ES
		30-40	30-40	30-40			90	90-100	95	90		ES
		20-30	20-30	20-30				100	100		peptide n	ES
												ES
							100	100	100	100	100	ES
		see pg 1					100					MALDI
		10-20	10-20	10-20	10-20	10-20	100	100	100	100	100	ES
		<10	<10	<10	<10	<10	100	100	100	100	100	MALDI
		10-20	10-20	10-20	10-20	10-20	54	54	100	100	24	ES
100 % in accordance with MS-seq			40-50	40-50	40-50		0	50	100	100		ES
		<10	10-20	10-20			50	100	100			ES
		30-40		30-40	40-50		100		100	100		ES/MALDI
			50-60	50-60				100	100			ES
				20-30			100	100		100	100	ES/MALDI
				10-20			100	100	100	100		ES
		10-20	10-20	10-20	10-20	10-20		100	100	100	100	ES
				40-50			100	100	100	88		ES/MALDI
											100	
									100	100		ES
		<10	<10	<10	<10	<10	100	100	100	100	30	ES
		90-100	90-10(	90-100		90-100		100				MALDI
		20-30	20-30	20-30	20-30	20-30	75	80	85	15	50	MALDI
		40-50	40-50	40-50	40-50	40-50	100	100	99	95		ES
							0	0	0	0		
							0	100	0	0	0	ES
		30-40					100					ES
							0	0		100		

MS ionization				Type of ES MS					Type of MALDI M
2	3	4	5	1	2	3	4	5	1
MALDI	MALDI	MALDI	MALDI						TTOF
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
ES	ES	ES	ES	LIT-FT	QTOF/LIT-FT	LIT-FT	LIT-FT	LIT-FT	
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
MALDI	MALDI	MALDI	MALDI						QTOF/TTOF/RTOF
ES	ES	ES	ES	LIT	LIT	LIT	LIT	FTMS/LIT	
MALDI	MALDI	MALDI	MALDI						RTOF/TTOF
MALDI	MALDI	MALDI	MALDI						TTOF
MALDI	MALDI	MALDI	MALDI						TTOF
ES	ES	ES	ES	LIT	LIT	LIT	LIT	LIT	
ES/MALDI	ES/MALDI	ES/MALDI	ES/MALDI	3DIT/RTOF	3DIT/RTOF	3DIT/RTOF	3DIT/RTOF	3DIT/RTOF	QTOF/xcxsc
ES/MALDI	ES/MALDI	ES/MALDI	ES/MALDI	LIT	LIT	LIT	LIT	LIT	QTOF
MALDI	MALDI	MALDI	MALDI						TTOF
MALDI	MALDI	MALDI	ES/MALDI					QTOF	TTOF
ES	ES	ES	ES	3DIT/QTOF	3DIT/QTOF	3DIT/QTOF	3DIT/QTOF	3DIT/QTOF	
MALDI	MALDI	MALDI	MALDI						TTOF
MALDI	MALDI	MALDI	MALDI						TTOF
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
ES	ES	ES		QTOF	QTOF	QTOF	QTOF		
ES	ES	ES	ES	3DIT/QTOF	3DIT/QTOF	3DIT/QTOF	3DIT/QTOF	3DIT/QTOF	
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
									TTOF
ES/MALDI	ES/MALDI	ES/MALDI	ES	QTOF	3DIT/QTOF	3DIT/QTOF	3DIT/QTOF	3DIT	
MALDI	MALDI	MALDI	MALDI						TTOF
ES	ES	ES	ES	3DIT	3DIT	3DIT	3DIT	3DIT	
ES	ES	ES		QTOF	QTOF/LIT-FT	QTOF/LIT-FT	QTOF/LIT-FT	QTOF/LIT-FT	
ES	ES			QTOF	QTOF	QTOF			
	ES/MALLDI	ES/MALLDI		3DIT		3DIT	3DIT		QTOF/RTOF
ES	ES	ES		QTOF	QTOF	QTOF	QTOF		
ES/MALDI	ES/MALDI	ES/MALDI	ES/MALDI	3DIT	3DIT	3DIT	3DIT	3DIT	RTOF
ES	ES	ES		LIT	LIT	LIT	LIT		
ES	ES	ES	ES	3DIT	3DIT	3DIT	3DIT	3DIT	
ES/MALDI	ES/MALDI	ES/MALDI		QTOF	QTOF	QTOF	QTOF		QTOF
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
ES	ES	ES	ES	QTOF	QTOF	QTOF	QTOF	QTOF	
MALDI	MALDI	MALDI	MALDI						RTOF/IT-TOF
MALDI	MALDI	MALDI	MALDI						TTOF
ES	ES	ES	ES	LIT	LIT	LIT	LIT	LIT	
ES	ES	ES	ES	LIT	LIT	LIT	LIT	LIT	
				3DIT					
		ES					QTOF		

S				Type of Derivatization	
2	3	4	5	1	2
TTOF	TTOF	TTOF	TTOF	SPITC/CAF/O-methylisourea/formyl N-acetylation	SPITC/O-methylisourea/form N-acetylation
QTOF/RTOF/TTOF	QTOF/RTOF/TTOF	QTOF/RTOF/TTOF	QTOF/RTOF/TTOF		O-methylisourea
RTOF/TTOF	RTOF/TTOF	RTOF/TTOF	RTOF/TTOF	CAF/methoxy dihydro imidazole	CAF
TTOF	TTOF	TTOF	TTOF		
TTOF	TTOF	TTOF	TTOF	O-methylisourea	O-methylisourea
3DIT/TOF	3DIT/TOF	3DIT/TOF	3DIT/TOF	imidazole (Lys-tag)	SPITC/imidazole (Lys-tag)
QTOF	QTOF	QTOF	QTOF		
TTOF	TTOF	TTOF	TTOF		18O incorporation
TTOF	TTOF	TTOF	TTOF	N-acetylation	N-acetylation
TTOF	TTOF	TTOF	TTOF		
TTOF	TTOF	TTOF	TTOF		
				CAF/gave no useful mass	
TTOF	TTOF	TTOF			
TTOF	TTOF	TTOF	TTOF		
	QTOF/RTOF	QTOF/RTOF	QTOF/RTOF		
RTOF	RTOF	RTOF	RTOF		
QTOF	QTOF	QTOF			
RTOF/IT-TOF	RTOF/IT-TOF	RTOF/IT-TOF	RTOF		SPITC
TTOF	TTOF	TTOF	TTOF		

3	4	5
SPITC/CAF/O-methylisourea/for	SPITC/CAF/O-methylisourea/formylation, ami	SPITC/CAF/O-methylisourea/formylation
CAF/methoxy dihydro imidazole	CAF	CAF
O-methylisourea	SPITC	SPITC
SPITC/O-methylurea/imidazole	SPITC/imidazole (Lys-tag)	SPITC/imidazole (Lys-tag)
18O incorporation N-acetylation	18O incorporation	18O incorporation
	SPITC/CAF	SPITC/CAF
SPITC	SPITC	SPITC

Type of Fragmentation					Mr		
1	2	3	4	5	1/T50 (1193.8349)	2/A2 (1396.6683	3/J1 (1463.74
HE-CID	HE-CID	HE-CID	HE-CID	HE-CID	1193.832	1396.669	1464.772
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.82	1411.64	1463.74
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.87	1395.68	1463.75
LE-CID	LE-CID/ECD	LE-CID/ECD	LE-CID/ECD	LE-CID	1192.82592	1395.65952	1463.76486
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.91	1395.76	1463.86
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.827	1411.654	1463.766
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.8275	1411.6611	1463.7661
HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	1192.838	1395.66	1463.78
HE-CID	HE-CID	HE-CID	HE-CID	HE-CID	1192.827	1395.661	1463.766
HE-CID	HE-CID	HE-CID	HE-CID	HE-CID	1192.8	1395.7	1463.8
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.6	1411.4	1463.5
LE-CID	LE-CID	LE-CID	LE-CID		1192.828	1395.6678	1463.7734
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.8	1395.6	1463.7
HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	1192.83	1396.64	1463.81
HE-CID	HE-CID	HE-CID	HE-CID	LE-CID/HE-CID	1192.8	1395.7	1463.8
	LE-CID	LE-CID	LE-CID	LE-CID		1395.668	1463.766
HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	1192.7	1395.7	1463.8
HE-CID/PSD	HE-CID/ISD	HE-CID/PSD	HE-CID/PSD	HE-CID/PSD	1192.8266	1395.65998	1463.76547
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.9	1411.7	1463.84
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.75	1411.69	1463.84
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.831	1395.716	1463.82
LE-CID	LE-CID	LE-CID	LE-CID		1192.7	1395.69	1463.82
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.83	1411.69	1463.84
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.85	1395.73	1445.82
HE-CID			HE-CID	HE-CID	1192	1395	1463
LE-CID	LE-CID/HE-(	LE-CID/HE-CID	LE-CID/HE-CID	LE-CID	1177.57	1411.8	1463.7
HE-CID	HE-CID	HE-CID	HE-CID	HE-CID	1192.8261	1395.6615	1463.761
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.2	1411	1463.8
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.86	1395.7	1463.8
LE-CID	LE-CID				1193.8185	1396.6825	
LE-CID/HE-CID/PSD		LE-CID/HE-CID/PSD	LE-CID/HE-CID		1194		1465
LE-CID	LE-CID	LE-CID	LE-CID		1364.82	1201.72	1463.87
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.828	1395.663 ;	1411463.782
LE-CID	LE-CID	LE-CID	LE-CID		1350.87	1411.89	1463.8
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.8	1395	1463
LE-CID	LE-CID	LE-CID	LE-CID		1073.58		1463.77
				LE-CID			
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	775.4	1515.6	1463.8
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1192.7775	1395.6159	1463.67
LE-CID/PSD	LE-CID/PSD	LE-CID/PSD	LE-CID/PSD	LE-CID/PSD	1192.67	1411.46	1464.09
HE-CID	HE-CID	HE-CID	HE-CID	HE-CID	1566.8	1395.78	1463.9
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1193	1396	1462
LE-CID	LE-CID	LE-CID	LE-CID	LE-CID	1193.7	1398	1480.8 or 146
LE-CID					1193.49		
			LE-CID		1192.85	1396.72	1411.7

4/A3 (1505.738	5/A1 (2328.141	Peptide Sequence (first choice) 1/T50 (LGAILKKLIPK)	core (11)
1505.748	2328.134	KLILQKLIPK	7
1504.73	2327.11	(L/I) G A (L/I) (L/I) K K (L/I) (L/I) P K	8.5
1504.72	2327.16	LGAILKIQIPK	9
1504.73058	2327.13351	(I/L)GA(I/L)(I/L)KK(I/L)(I/L)PK	8.5
1503.78	2327.2	XGA(I/L)(I/L)(Q/K)K(I/L)(I/L)PK	7.5
1504.742	2327.133	KHyp(L/I)(L/I)KK(I/L)(I/L)PK	6
1504.7301	2327.1345	(L/I)GA(L/I)(L/I)KK(L/I)(L/I)PK	8.5
1504.731	2327.133	LGALLKKLLPK	7
1504.731	2327.133	kllklqllpk	4
1504.7	2327.2	vvR(I/L)KKHypHypPK	4.5
1504.5	2326.8	(I/L)GA(I/L)(I/L)(K/Q)(K/Q)(I/L)(I/L)PK	7.5
1504.7324	2327.1451	(I/L)GA(I/L)(I/L)KK(hydrP)(I/L)PK	8
1504.7	2327.1	K(Hydr P)(I/L)(I/L)KK(I/L)(I/L)PK	6
1504.75	2327.16	KLLKPhydKLLPK	4
1504.8	2327.2	Q(I/L/hP)(I/L/hP)LKK(I/L/hP)(I/L/hP)PK	5
1504.74	2328.15	LXAILKKLIDL	7
1504.7	2327.1	ga(l/i)(l/i)psgag(l/i)(l/i)pk	6
1504.73047	2327.13297	KL(L/I)K(I/L)K(I/L)PohPK	2
1504.84	2327.37	(K/Q)(I/L)(I/L)(I/L)(K/Q)(K/Q)(I/L)(I/L)PK	2
1504.71	2331.09	(I/L)(QK)(I/L)(I/L)(QK)(QK)(I/L)(I/L)PK	5
1504.811	2327.139	KLLKLGALLPK	2
1504.74		(I/L)GA(I/L)(I/L)(Q/K)(I/L)AG(I/L/hP)PK	6
1504.74	1177.58	(I/L)GA(I/L)(I/L)(K/Q)(K/Q)(I/L)(I/L)PK	7.5
1504.77	1411.72	(I/L)GA(I/L)(Q/K)(Q/K)(I/L)(I/L)PK	7
1504	2327	(Q/K)(I/L)RVVK(I/L)(I/L)P(Q/K)	3.5
1504.8	1555.2	TFNMsxGQHS(I/L)K	1
1504.7299	2327.107	KLLLKKLP*PK	2
1504.7	2327	LGAIK(467.2)PK	6
1504.8	2327.3		
		(242.6)(I/L)(Q/K)(I/L)(Q/K)(I/L)(356.5)	0
1506.5		(Q/K)Poh(I/L)(Q/K)(Q/K)(I/L)(I/L)PK-NH2	4.5
1504.84		aygplvpvslppr	0
1504.74	2327.143	KD(i/l)(i/l)qkasyK	1
1504.88		VY(Q/K)APS(L/I)SAPYR	0
1504	2327	lgalIKGA(L/I)(L/I)PK	5
1504.7		(I/L)PASHypSPVYK	1
	2327.2		
1504.6	740.2		0
1504.6846	2327.0728	LGABBQQBBPK	5
1504.92	2327.2	(243.09)(I/L)(I/L)(126)DK(355.36)	0
1504.88 minus n	2327.35	(RD)(P-Q/K)L(F/Msx)YEAN(315.01)	0
1080	1350	(K/Q)hydroxyP(I/L)(I/L)G(K/Q)A(hydroxyP)(I/L)PK	0
		VXKPLAK(hydroxypro)IPVN	0
1412.8	1480.8		
		KN(I/L/Hyp)	0
1463.82	1504.83		
		V Y K P hydP A S hydP S P V Y K(K)	0

2/A2 (AYTFN MGQHSLK)	core (12)	3/J1 (VYKPHypASHypSPVYK)	core (13)
AYTFNMGQHSLK	12	VYKPhypAShypSPVYK	13
AYTFN Msx GQHS(L/I)K	11.5	VYKP(Hyp)AS(Hyp)SPVYK	13.0
AYTFNMGQHSLK	12.0	VYKP(Hyp)AS(Hyp)SPVYK	13.0
AYTFNMGQHS(I/L)K	11.5	VYKPPoxASPoxSPVYK, Pox=hydroxyproline	13.0
AYTFNMGQHS(I/L)K	11.5	VYKP(I/L/x)ASXSPVYK; X=hydroxyproline	12.3
AYTFNMsxGQHS(L/I)K	11.5	VYKPHypASHypSPVYK	13.0
(AY)TFNMsxGQHS(L/I)K	11.5	VYKP(L/I)AS(L/I)SPVYK	11.0
AYTFNMGQHSLK	12.0	VYKP(ps)S(tv/ea/cp/sl)(qc)qK	5.0
YATFNMGQHSLK	9.0	VYKPP(OH)ASP(OH)SPVYK	13.0
(AY)TFEGMLHSLK	8.0	vykplaslspvyk	11.0
(AY)TFNMsx(K/Q G)HS(I/L)K	9.5	FD(K/Q)P(I/L)AS(I/L)SPVYK	7.0
AYTFNMGQHS(I/L)K	11.5	VYKP(hydrP)AS(I/L)SPVYK	12.0
YATFNMGQHS(I/L)K	9.5	K(134)KP(I/L)AS(I/L)SHS(CysP)K	5.0
HPTFNMGQHSPhydK	9.0	VYQPLASLSPVYK	9.0
PHTFNMGQHS(i/l)K	9.5	VYKPIAShPSPVYK	12.0
AYTFNMGQH(L/I)SK	10.0	VYKPHypASHypSPVYK	13.0
ag(l/i)spgvsm(l/i)hpck	1.0	vy(k/q)p(l/i)apcpsvyk	7.0
YATFNMGQHSLK	10.0	VYKPPohASLSDFPK	8.0
AYTFNMsxG(K/Q)HS(I/L)K	11.0	VY(Q/K)P-hyp-AS(l/hyp)SPVYK	12.0
AYTFNMsxG(Q/K)HS(I/L)K	11.0	VYKP(I/L)AS(I/L)SPVYK	11.0
AYTFNMGKHSLK	11.0	VYKPASLSPVYK	11.0
YATFNMG(Q/K)HSLK	9.5	VYK P(I/L)AS(I/L)SVPYK	11.0
AYTFNFG(K/Q)HSHypK	10.5	VY(K/Q)P(I/L)ASs(i/l)PVYK	9.5
AYTFNMG(K/Q)HS(L/I)K	11.0	(YV)(K/Q)P(I/L)AS(279.12)VYK	8.5
HPTFNMG(Q/K)HS(I/L)(Q/K)	8.5	PHGVPIASPCPVY(Q/K)	3.5
AYTFNMsxGQHS(I/L)K	11.5	VYQP(I/L)AS(I/L)SpvYK	10.0
PHTFNMGKHSP*K	1.0	AYRPIASISPVYK	5.0
(221.0)PA(427.0)NFTSK	1.0	VYKPHypASHypGPKYK	10.0
AYTFNM	6.0	VYKPLASHypSPVYK	12.0
AYTFNMGQHSLK	12.0	VYKP(I/L)AS(I/L)SHDPR	6.0
		vVYKPPohASPohSPVYK	13.0
agplascppvyk	2.0	VYKPLASLSPVYK	11.0
AYTFNMGQHS(I/L)K	11.5	cmTFNkgfhsLK	1.0
(235)T(Msx/F)(114)(Msx/F)(185)	0.0	VY(Q/K)P(I/L/P-OH)AS(200)PVYK	9.8
(L/I)(Q/K)SHPSMNFTSK	1.0	(649)vasapqdk	1.0
		VY(K/Q)PHypASHypSPVYK	12.5
	0.0	VYKP(L/I)ASHCRYK, and some oxidized form @Cys resid	8.0
FSTFBFAVHTVK	1.0	YVKPMSSABPBMK	3.0
(145.09)DSH(I/L)(I/L)GQF(336.44)	0.0	(263.13)KP(I/L)ASH(422.82)K	6.0
(YA/FS/HP/MC)T(Msx/F)NMG(Q/K)H(EA/TV/C	4.5	(262.32)(Q/K)P(LA/PS)S(TV/CD/LS/EA)(487.19)	0.0
YATFNMNAS(I/L)K	6.5	VQNNMoxYEANPR	1.0
AYTF(Hydroxypro)MIFHXLykr	5.0	LXAIAKSLSEA	0.0
FSTFNMSYASMK	1.0		

				Total Score (70)
4/A3 (GVPGADIFYEANPR)	**Score (14)**	**5/A1 (FPHVANSGEWPDLVYVVNER)**	**Score (20)**	
GVPGADIFYEANPR	14	FPHVANSGEWPDLVYVVNER	20	66
GVPGAD(L/I)FYEANPR	13.5	F P H V A N S W W P D (L/I) V Y V	17.5	64.0
GVPGADIFYEANPR	14.0	FPHVANSWWPD(I/L)VYVV(K/Q)DR	16.0	64.0
GVPQD(I/L)FYEANPR	13.5	(Mh(i/l))VANSGEWPD(I/L)VYVVNER	16.5	63.0
GVPGADXFYEAGGPR	12.0	FPHVANSGEWPDXVYVVNER	19.0	62.3
RPGAD(L/I)FYEANPR	11.5	FPHVANSGEWPD(L/I)VYVVNER	19.5	61.5
rpQD(L/I)FYEANPR	8.5	FPHVANSGEWPD(I/L)VYVVNER	19.5	59.0
GVPGADIFYEANPR	14.0	FPHVANSGEWPDLVYVVNER	20.0	58.0
GVPGADLFYEAGGPR	12.0	FPHVANSGEWPDLVYVVDQR	18.0	56.0
GVPGADLFYEANPR	13.0	FPHVANSTTPDLVYVVG(GE)R	16.0	52.5
RPGAD(I/L)(F/Msx)YEANPR	10.5	(I/L M)HVANSGEWPD(I/L)VYVVNER	17.5	52.0
GVPGAD(I/L)FYEANPR	13.5	F(791.9)W(212.1)LVYVV(243.1)R	7.0	52.0
VGPGAD(I/L)FYEANPR	11.5	FPHVANSGEADPD(I/L)VYVVNER	18.5	51.5
RPGADLFYEANPR	11.0	FPHVANSWWPDLVYVVNER	18.0	51.0
RPGADIFYEANPR	12.0	(568.3)PSWWPD(I/L/hP)VYVVNER	10.3	48.8
(VG)PGADIFYEANPR	12.0	(235.19)fv(214.05)pfw(212.15)(I/L/I	5.3	48.3
GVPGADIFYEANPR	14.0	FPHvanswadpd(l/i)vyvvnER	16.5	44.5
GVDNAEYFLAGPPR	4.0	FPHVANSGEWPDLVYVVDQR	18.0	42.0
rP(K/Q)DLFyEAnpR	7.0	(938.57)WpdLVYVV(243.14)R	9.0	41.0
GVPGAD(I/L)FYcgPGPR	11.0	(CS)(I/L)NVVYV(I/L)DP(1110.5)	3.0	41.0
GVPSLRFYEPGKR	7.0	PDLVYGFFWPDLVYVVGWR	9.0	40.0
GVPGAD(I/L)FYEANPR	13.5			40.0
RPGAD(I/L)FYEAggPR	10.5	TFNFg(k/q)HSHypK	0.0	38.0
VGPGAD(I/L)FYEANPR	11.5	AYTFNMsxG(Q/K)HS(L/I)K	0.0	38.0
GVPGADIFYEANPR	14.0	FNFASEGWWLVLVYVVRDK	5.0	34.5
(156)PGAD(I/L)FYEANPR	11.5	T(I/L)(I/L)VNGVMYF(400)	0.0	34.0
RP(GAD)IFYEANPR	12.0	EDHVANSVSVSP*VP*VYVVNER	12.0	32.0
GVPGADIFYEANPR	14.0	(678.2)t(424.1)wegs(i/l/hyp)av(381	0.0	31.0
(VG)PGADIFYEANPR	12.0			30.0
RP(Q/K)D(I/L)FYEANPR	9.5	(?)VVYV(I/L)DPW(?)	2.0	29.5
RPQD(I/L)FYEANPR	8.5			26.0
gvpgadlfyeaggpr	12.0			25.0
RPQD(I/L)FYEANPR	8.5		0.0	22.0
RPGAD(I/L)FYEANPR	11.5			21.3
RPQD(I/L)FYEANPR	4.0	RPQD(I/L)FYEANPR	8.5	19.5
PQDLFYEAGGPR + neutral loss of 157	6.0			19.5
		FPHVANSGEWPD(I/L)VYVVNER	19.5	19.5
RPGAD(L/I)FYEAGGPR	10.5			18.5
RPGADBFFNFHGK	4.0	(1449.6094)VYVVGWR	5.0	18.0
GVDRNAGSYFA(I/L)PR	4.0	F(2005.2)R	2.0	12.0
(Q/K-P)DL(F/Msx)YEANPR	6.0	(YA/FS/HP/MC)(F/Msx)AYYVLDPW(920.5	0.0	10.5
ED(I/L/hydroxyP)(K/Q)TNHPK	1.3	MoxDQP(hydroxyP)ASAEDEDK	0.0	8.8
SPLVNDGQEXK	0.0			5.0
				1.0
				0.0
(VY)(Q/K)PSP(I/L)S(PV)Y(K/Q)	0.0			0.0
				0.0
				0.0

Peptide Sequence (second choice)

1/T50 (LGAILKKLIPK)	**2/A2 (AYTFN MGQHSLK)**	**3/J1 (VYKPHypASHypSPVYK)**	**4/A3 (GVPGADIFYEANPR)**
	AYTFNMsxGQHSLK		
	also oxidized form, Msx		X=hydroxyproline
LGALLKK(hyP)(hyP)PK	AYTFNMGQHS(L/I)K	VYKP(l)AS(l)SPVYK	gvpQD(L/I)FYEANPR
K(I/L)(I/L)Q(I/L)K(I/L)(I/L)PK K(I/L)(I/L)(I/L)KK(I/L)(I/L)PK			
		VYKAPSLSPVYK	
(I/L)GA(I/L)(Q/K)241.19PK (Q/K)(Q/K)(I/L)(I/L)(I/L)(Q/K)	AYTFNMG(Q/K)HS(I/L)(Q/K)		
KLLLKKP*LPK	(FT)SFNMGKHSP*K	PHGVPIASISPVYK	VGPGADIFYEANPR
	HSLK		
(nf)(i/l)sf(L/I)(L/I)PK			
LGABBQAGBBPK	HPTFBFAVHTVK, B is Hydroxypr	YVGAPMSSABPLMK	RPGADBFFNFGHK

	Peptide Sequence (third choice)			
5/A1 (FPHVANSGEWPDLVYVVNER)	**1**	**2**	**3**	**4**

Met likely present - we see a minor peptide ~16u bigger
X=hydroxyproline

(IM)HVANSGEWPD(I/L)VYVVNER				
FPHVANSGEWPD(hyP)VYVVNER	GLALLKKLLPK			
FPHVANSGEADPD(I/L)VYVVGGER				
	(I/L)GAhydroxyprolineK(Q/K)(I/L)(I/L)PK			
	K(LLP*)KKLLPK	CMTFNMGKHSP*K	RPHPIASISPVYK	
	ava(L/I)(L/I)qga(L/I)(L/I)PK			
(1202.6210)W(920.3619)			YVGAPMSSABPBMK	RPGADBFFNFPPK
VQD(I/L/hydroxyP)MoxYEANPR				

5	Type of amino acid composition information 1	2
	II/MD/WI	II/MD/WI
	II/MD	II/MD
	II/MD	II/MD
H and I/L deduced from accurate mass fit	MD	MD
	II	II
	MD	MD
	MD	II/MD
	MD	
	II	II/MD
	II	II
	MD/MS3	MD/MS3
	MD	MD
	II/MD	II/MD
	II/MD	II/MD
	II/MD/WI/N-acetylation resolved K/Q ambiguity	II/MD/Analog with Msx a
	II/MD	MD
	II/MD/WI	II/MD
	II/MD	II/MD
	II	II/MD
	MD	MD
	II/MD	II/MD
		II/MD
	II/MD	II/MD
	II/MD/+2 fragment ions/internal fragments	II/MD/peptide parent io
	II/MD/IF	II/MD/IF
	II/MD	II/MD
	II	II
	II/MD	
	MD/internal acyl ions	MD
	MD	MD
(1449.6094)VYVVWGR(-0.0176)	II	II
		SPITC
	II/MD	II/MD
	MD	MD
	MD	MD

3
II/MD
II/MD
II/MD
MD
II/MD
MD
MD
II
II
II
MD/MS3
MD
II/MD
II/MD
II/MD/w-ions discriminate I/L at residue 5; intensity of y6 suggests hPro at
MD
II/MD
II/MD
II
MD
II/MD
II/MD
II/MD
II/MD/+2 fragment ions/internal fragments
II/MD/internal @1202 differentiates given seq from alternatives. NOTE: w9 io
II/MD
II

II

II/MD

MD
MD

II

II/MD
MD

MD

4	5	Mass di 1
II/MD/w ions	II/MD/w ions	GA vs.
II/MD	II/MD	
II/MD	II/MD	
MD	MD	GA ~ Q
II	II	
MD	MD	
MD	II/MD	
II	II	
II/MD	II/MD	
MD/MS3	MD/MS3	
MD	MD	GA vs K
II/MD	II/MD	
II/MD	II/MD	
II/MD/w-ions discriminate I/L at residue 6; y8 intensity attributed to D a	II/MD	
MD	II/MD	
II/MD	II/MD	128
II/MD	II/MD	
II	MD	
MD	II/MD	
II/MD		
II/MD		
II/MD	II/MD	
II/MD/+2 fragment ions/internal fragments	II/MD/+2 ragment ions/interna	128.1
II/MD/internals. NOTE w8 shows I not L		
II/MD	MD	
II	II	
II		
II/MD		
MD	II/MD	
	II/MD	
II	II	
	II	
MD	II/MD	
MD	MD	
MD	MD	
II/MD		

fferences for dipeptide or single amino acid

2	**3**	**4**	**5**
K			
N for (GG)			N and GG
			IM/FP
		128 = Q OR GA (very weak G)	
EG = W			
	FD=(YV)	R=(GV)	(I/L M)=(D/E)=(F/Ms
AG vs Q			
	369.1 PLAS inter	341.1 PGAD internal ion	
114	114	114	114
128	128	128	
			GE=W
K =AG			
	156		
C-term (Q/K) mightbe GA			
		RPGAD(I/L)FYEANPR	
		GA = Q, K	

Directionality (N terminus to C terminus) deduction
1
Identified y1 ion
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Identified yn ion and yn + 115Da (Asp)/I
Identified y1 ion/Identifined yn ion and bn ion
Identified bn ion and a corresponding ion at -28Da/assumed tryptic peptide, looked for bn
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/complete series of b and y ions
correlation of y and b series ions
Identified y1 ion/In one instance a full b-ion series!
Identified yn ion and yn - 97Da (Pro)/P-induced internal fragments
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
sequence consistency and MS3
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified bn ion and a corresponding ion at -28Da/Identified bn ion and a corresponding ion at -28Da/y+b=MH+
Identified y1 ion
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da

Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Identified yn ion and yn - 97Da (Pro)
Identified y1 ion
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified yn ion and yn - 97Da (Pro)
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/peptide parent ion mass
Identified bn ion and a corresponding ion at -28Da
Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (Pro)
Identified y1 ion
in combination with Edman

Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Identified bn ion and bn +18Da
Identified bn ion and bn +18Da
Based on 'bn' ions

Identified bn ion and a corresponding ion at -28Da
Identified y1 ion

Identified y1 ion
Identified y1 ion
y and b ion pair
Identified bn ion and a corresponding ion at -28Da

Identified bn ion and a corresponding ion at -28Da/high mass b-ion

2

Identified y1 ion/intuitive
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified yn ion and bn ion
Identified bn ion and bn +18Da/assumed tryptic peptide, located bn
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/complete series of b and y ions
correlation of y and b series ions
Identified y1 ion
automated, P-induced internal fragments
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
sequence consistency and MS3
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified bn ion and a corresponding ion at -28Da/Identified bn ion and br
Identified y1 ion/18O incorporation
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Absen
Edman sequence
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion
Identified bn ion and a corresponding ion at -28Da
Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (Pr
Identified y1 ion/
Edman, but I'm really not sure of directionality for this peptide
Identified bn ion and a corresponding ion at -28Da

Identified bn ion and a corresponding ion at -28Da/Identified bn ion and br
Based on 'an' and 'bn' ions

Y11 ion

Identified y1 ion

Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/y and

high mass b-ions

3

Identified y1 ion/intuitive
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Ide
Identified y1 ion/Identified bn ion and yn ion
Identified bn ion and bn +18Da/assumed tryptic peptide, located bn
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/complete series of y ions
correlation of y and b series ions
Identified y1 ion
Identified bn ion and bn +18Da/Identified bn ion and bn +18Da/Identified
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Ide
sequence consistency and MS3
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified bn ion and bn +18Da/y+b=H++1
Identified y1 ion/18O incorporation
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Edman
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Ide
Identified y1 ion/Identified yn ion and yn - 97Da (Pro)
Identified y1 ion
Identified y1 ion
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Identified y1 ion/Identified yn ion and yn - 97Da (Pro)
peptide parent ion mass

Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (
Identified y1 ion
in combination with Edman

Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Ide
Identified bn ion and a corresponding ion at -28Da/Identified bn ion and
Based on software interpretation

Identified y1 ion/Identified bn ion and a corresponding ion at -28Da

Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (
Identified y1 ion
Identified y1 ion
I guessed that the large ion which ends in P is a y ion

high mass b-ion

4
Identified y1 ion/intuitive
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Identi
Identified y1 ion/Identified bn ion and yn ion
bn ion +18Da/assumed tryptic peptide, located bn
Identified y1 ion
Identified y1 ion/Complete series of b and y ions
correlation of y and b series ions
Identified y1 ion
Identified yn ion and yn + 115Da (Asp)/y-y17, b-a, P-induced internal fragm
y-ions, because of the spitc derivatization
sequence consistency and MS3

bn +18Da
Identified y1 ion/18O incorporation
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
Edman
Identified y1 ion
Identified y1 ion
Identified y1 ion

Identified y1 ion
Identified yn ion and yn - 97Da (Pro)
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da
peptide parent ion mass

Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (Pro
Identified y1 ion
in combination with Edman

bn +18Da
Identified bn ion and bn +18Da
Based on software interpretation

Identified y1 ion

Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (Pro

Identified y1 ion

high mass b-ions

Identified y1 ion

	Accurate mass measurements					Mass
5	**1**	**2**	**3**	**4**	**5**	**1**
Identified y1 ion/intuitive	Y	Y	Y	Y	Y	5-10
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Identified yn ion and yn +	Y	Y	Y	Y	Y	>10
Identified y1 ion	N	N	N	N	N	5-10
Identified bn ion and bn +18Da/assumed tryptic peptide, located bn	Y	Y	Y	N	Y	<1
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da	Y	Y	Y	Y	Y	>10
Identified y1 ion	Y	Y	Y	Y	Y	1-5
Y and B series correlation	Y	Y	Y	Y	Y	1-5
Identified y1 ion	Y	Y				5-10
Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (Pro)/P-induced internal	Y	Y	Y	Y	Y	1-5
y-ions, because of the spitc derivatization						
sequence consistency and MS3						
	Y	Y	Y	Y	Y	1-5
Identified bn ion and a corresponding ion at -28Da/Identified bn ion and bn +18Da/y+b=MH++1	Y	Y	Y	Y	Y	5-10
Identified y1 ion						
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da						
Identified y1 ion		Y	Y	Y	N	1-5
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da	Y	Y	Y	Y	Y	>10
Identified y1 ion/Identified bn ion and a corresponding ion at -28Da/Identified yn ion and yn +	Y	Y	Y	Y	Y	5-10
Identified bn ion and a corresponding ion at -28Da						
	N	Y	Y	N		
	Y	Y	Y	Y	Y	5-10
			Y	Y		
Identified y1 ion						
Identified y1 ion/peptide parent ion mass						>10
Identified y1 ion						
Identified yn ion and yn + 115Da (Asp)/Identified yn ion and yn - 97Da (Pro)	Y	Y	Y	Y	N	5-10
Identified y1 ion	Y	Y	Y	Y	Y	5-10
Edman, but I'm really not sure of directionality for this peptide	Y	Y	Y	Y	Y	>10
bn +18Da	Y		N			1-5
Based on 'bn' ions and software denovo sequencing results	Y	Y	Y	Y	Y	5-10
		Y11 ion				
	Y	Y	Y	Y		5-10
Identified y1 ion						
)			Y	Y		
	Y	Y	Y	Y	Y	5-10
Identified y1 ion						
Identified y1 ion/identified a possible b ion						
Identified bn ion and a corresponding ion at -28Da						
Identified bn ion and a corresponding ion at -28Da/high mass b-ion						

Accuracy				Accurate Mass Information Used				
2	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
5-10	5-10	5-10	5-10	PAR	PAR	PAR	PAR	PAR
>10	>10	>10	>10	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD
5-10	5-10	5-10	5-10					
	<1	<1	1-5	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD
>10	>10	>10	>10	PAR	PAR	PAR	PAR	PAR
1-5	1-5	1-5	1-5	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD
1-5	1-5	1-5	1-5	PAR	PAR	PAR	PAR	PAR
1-5		1-5	1-5	PAR	PAR		PAR	PAR
1-5	1-5	1-5	1-5	PROD/PAR	PAR	PAR/PROD	PAR	PAR/PROD
1-5	5-10	1-5	5-10	PAR	PAR	PAR	PAR	PAR
5-10	5-10	5-10	5-10	PROD	PROD	PROD	PROD	PROD
		1-5	1-5		PAR	PAR	PAR	PAR
>10	>10	>10	>10	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD
5-10	5-10	5-10	5-10	PAR	PAR	PAR	PAR	PAR
>10	<10				PROD	PROD		
5-10	5-10	5-10	5-10	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD
	1-5	1-5				PAR	PAR	
>10	>10	>10	>10					
5-10	5-10	5-10	>10	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD	
5-10	5-10	5-10		PAR	PAR	PAR	PAR	PAR
>10	>10	>10	>10	PAR	PAR	PAR	PAR	PAR
				PROD				
	5-10							
5-10	5-10	5-10	5-10	PAR	PAR	PAR	PAR	PAR
5-10	5-10	5-10		PROD	PROD	PROD	PROD	
5-10	5-10	5-10				PROD	PROD	
5-10	5-10	5-10	5-10	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD	PAR/PROD

Software used to aid in the determination of the peptide sequences	Software Version
Manual/in house software, Protein Prospector	0.0, 4.0.4
Peaks	2.4
Manual	
Manual/sequenced manually	
BioAnalyst/Manual/ProMaC 2.0	1.1
TOFMA	-
DeNovoX/DeNovoX 2	alpha version
BioTools/Manual	BioTools 2.2
BioTools/RapiDeNovo	BT 3.0, RDN 3.0
Manual/Data Explorer	version 4.4.
DeNovoX	1
SpectrumMill	A.03.01.037
BioAnalyst/MassSeq	Analyst 1.4 and Masslynx 4.0
BioTools	2.2
Manual/DeNovo Explorer	2
BioTools	3
BioTools/Manual	2.2
Manual/If 'Manual' means manual, then that's wha I used, as well as the Pep	4.0 for MassLynx
BioAnalyst/Manual	
MassSeq	3.5
Manual/MassLynx (Interpret)	4
MassSeq/Peaks	MassSeq (Masslynx v4.0sp2), Pe
Manual/Home made + ABI de novo explorer	most recent of each
BioAnalyst/Manual	Not sure
Denovo Explorer on 4700 Proteomics Analyzer/Denovo Explorer on 4700 Proteom	Version 3
Micromass BioLynx	MassLynx 3.50
Manual	
Peaks	2.2
Peaks	
BioAnalyst/Manual	Analyst 1.4
Peaks	2.4
BioAnalyst/Manual/Peaks	Bioanalyst 1.1.1, PEAKS 2.4 s
BioAnalyst/Manual/SpectrumMill	A.03.01.032
Manual/Peaks	PEAKS 2.0
BioAnalyst	1.1
Manual	
Data Explorer	
Peaks	2.4
Manual	
Manual	
Manual	Data analysis program 610A

MS/MS database search algorithı	Manual Verificatio	Strategy used for interpretation
N	Y	I used software to aid in my manual interpre
N	Y	I used software to aid in my manual interpre
N		
N		
N	Y	I used software to aid in my manual interpre
N	Y	I used software to aid in my manual interpre
N	Y	I used software to aid in my manual interpre
N	Y	I used both but consider my manual interpret
N	Y	I used software to aid in my manual interpre
N	Y	I used software to aid in my manual interpre
N	Y	I used software to aid in my manual interpre
N	Y	I used both but consider my manual interpret
Mascot	Y	I used both but consider my manual interpret
N	Y	I used both but consider my manual interpret
N	Y	I used both but consider my manual interpret
N		
N		I used software to aid in my manual interpre
N	Y	I used both but consider my manual interpret
N	Y	I used software to aid in my manual interpre
N	Y	I used software to aid in my manual interpre
N	N	I used both but consider my manual interpret
N	Y	I used both but consider my manual interpret
Mascot	Y	
Mascot		
N	Y	I used both but consider my manual interpret
N	Y	I used software to aid in my manual interpre
N	N	
BLAST/Prowl/Sequest		
N	Y	I used both but consider my manual interpret
N		
N	N	
N	N	I used both but consider my manual interpret
N	Y	I used software to aid in my manual interpre
N	Y	I used both but consider my manual interpret
N	Y	I used both but consider my manual interpret
N	Y	I used both but consider my manual interpret
BLAST	Y	I used both but consider the software output
N	N	
N		
N		I used both but consider my manual interpret
N	N	
N		
N		
BLAST	Y	I used both but consider my manual interpret

If you manually interpreted the spectra and also used software to assist in your interpretation, do you consider the

N
N

Y
N
Y
N
Y
tation
Y
N
N
N
N

N
Y
N
Y
N
N

N
N

Y

N
N
N
Y
N
N

N
N

Y

If you manually interpreted the spectra and also used software to assist in your interpretation, how many hours

1-4
<0.5

1-4
0.5-1
1-4
0.5-1
1-4
1-4
0.5-1
1-4
1-4
0.5-1
1-4

1-4
0.5-1

<0.5
1-4
1-4

1-4
<12

0.5-1

0.5-1
1-4
0.5-1
1-4
1-4
0.5-1

0.5-1

<0.5

If you manually interpreted the spectra without using software to assist in your interpretation, how long

1-4
0.5-1
1-4
>12

1-4
<12
<0.5
<12

1-4
1-4
1-4
0.5-1
1-4
1-4

0.5-1

0.5-1

1-4

0.5-1

1-4

0.5-1

I tried but I gave up
0.5-1

0.5-1

1-4

0.5-1
<0.5

How would you rate the difficulty level of th:

doable
challenging
doable
challenging
challenging
doable
challenging
challenging
challenging
challenging
challenging
doable
challenging
challenging
challenging
doable
challenging
doable
doable
doable
doable
challenging
easy
challenging
challenging/The committee are sadists. Genera:
challenging
easy
challenging
challenging
challenging
challenging
doable
doable
challenging
challenging
challenging/and we ran out of time to commit t
Too tough for me.
doable
doable
challenging
challenging
doable

challenging

doable
challenging

Do you think this study has been useful?
Open-Ended Response
This study was very helpful in developing skills in de novo analysis and various derivatization protocols.
Yes
Yes
yes
Yes, after receiving the peptide sequences.
yes
Quite useful to benchmark current capabilities. The submission form, though, is inadquate for such a complex issue to be done by
Yes, indeed!! It has stimulated my group to many fruitful discussions, and forced us to learn new methods on the TOF/TOF we use.
It was useful to test the quality of software and the operators level of experience. However, it was an artificial example that
yes, and I'm looking forwards to see the 'right' sequences.
Yes
yes. It was useful to use a variety of approaches, including chemical mass tags and being able to compare the results internally.
Yes. It allowed us to learn in more detail the intricacies of de-novo sequencing. We are anxiously looking forward to learning
It showed us that de-novo-sequencing software is not yet ready to deal with challenging samples without manual assist.
Need to see the results before deciding. But, we are impressed with the Applied Biosystems 4700 TOF/TOF to produce high-quality
Yes. In this lab the combination of Edman, MALDI-TOF of the intact peptides and LC-MS/MS were essential together in getting the

Yes, especially for beginners as a way to practise. A little bit problematic were the high numbers of ambiguities, especially in
A good exercise for me since I don't often do de novo sequencing, but very time-consuming. The usefulness will be determined wher
Definitely
IT HAS BEEN USEFUL
Yes, I think so.
Yes
yes
Yes, It's help my skill level and pointed out failures of my present approaches too de novo sequencing.
We will see!
Yes.
Absolutely, and it was fun too. I think that it would provide a good tool for lobbying for more sensitive equipment.
Interesting, probably needs more practice

Yes, but unfortunatly had to be done low priority therefore was not complete.

Yes. I would have liked to have spent more time with additional strategies (e.g. derivatizations, digests) to get more complete
Yes. Despite the study is far away from the daily work it provides a type of self-improvement and gives the opportunity to 'pla
Yes, It should show that there needs to be some more work on software packages for denovo
Yes, I wouldn't have spent so much effort on de novo sequencing without this challenge. Know I will learn more when the results
not much. Sequences are known or homologoue exist in real life samples and can be solved much easier. It took too long than exp
YES

yes
yes. this has truly challenged my abilities in de novo sequencing, and I believe that I will learn a lot when we discuss the res
Yes, this study has been useful in teaching me how to do denovo sequencing. We did not have any projects that allowed me to do so

Yes!

yes
Yes. I was looking forward to analyzing the peptide mix you sent me. Unfortunately, my Q-Tof-2 mass spectrometer was down for fou

What type for study would you like to see the ABRF Proteomics Research Group conduct in the future?
Open-Ended Response

PTM analyses

The analysis of a complex mixture of proteins (greater than 500) and the assessment of criteria groups use to identify proteins (

We would like to challenge to identify many kinds of posttranslational modifications systematically.

quantitation

More complex protein mixture with PTM for identification/characterization.

more of those

Similar, but with emphasis on PTM's

-Glycopeptide analysis -2D-Gel spot/ 1D PAGE band analysis with PTM detection -isolated protein (recombinant?) full covalent st

Identification of PTMs

generic PTM study

Glycosylation and or Arginine methylation.

-

spectra suitable for de novo sequencing.

PTH analysis in a real sample. Quantitation of PTMs. Example, degree of phosphorylation. Degree of acetylation and methylatior

Identification of various PTM's in peptides to get an idea how those peptides behave (e.g. N-term acetylation, myristoylation, su

the data are compiled.

Identification of proteins in complex mixture, or identification of phosphorylated sites in protein

more ptm analysis

a 'reasonable' size protein mixture (10 or fewer) at reasonably similar concnetrations that must have all components identified.

Protein ID (Especially low abundant proteins) from Complex Mixture

I think this study was right up my alley, and was really useful for me. I'd like to see something similar.

I would like to see this type of study again. I would also like to see a protein ID study. We were not aware of the arrival c

The (relatively) quantification of peptides by mass spectrometry is despite the high level of the equippment still very unexact.

You guys do a good job of coming up with challenges--I think almost any exercise, as long as it is not so difficult that only a f

ected.

Not sure

quantitative studies

ults.

before this.

An in gel digest of a protein mixture (2-3 proteins)

r weeks and I could not analyze the sample. I was also away on a trip to Boston for a week before my Q-Tof-2 went down. Please ac

(Sequest, Mascot scores, false positives) plus an evaluation of the methods (Mudpit vs gel based). Quantitation study also,

ɔructure analysis

ı in a histone.

ılfatation; generally PTM's which are less common).

Sample should be provided as a mixture of intact molecules therby leaving open the option of preseparation or MUDPIT.

ɔf the sample set for the present study, such that the peptides sat at room temp for about 1 month. We only had about a week t
Would it be worth a study to find out if there are strategies to minimize the error?

ɛew labs can get the result, is worthwhile. Sorry, I'm not more specific. But thanks for your work.

ɔcept my apologies. I look forward to participating in your future studies.

e.g 2 samples containing the same 20 or so proteins at different levels (some equal).

.o work on our results.