# Error Tolerant Searching of Uninterpreted MS/MS Data

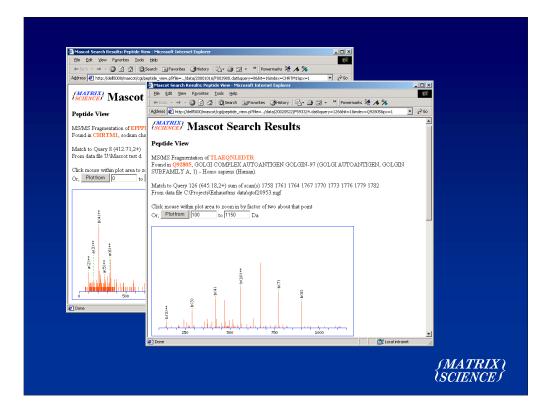


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User	: JSC	
Email	: jcottrell@ma	trixscience.com
Search title	: Annexin firs	t pass
MS data file	: C:\Projects\	Exhaust\ms_data\gtof20953.mgf
Database	: MSDB 2002041	5 (851746 sequences; 265326103 residues)
Timestamp		at 15:35:36 GMT
Significant hit		HUMALPPA NID: - Homo sapiens
	<u>Q96DB7</u>	HYPOTHETICAL 58.0 KDA PROTEIN Homo sapiens (Human).
	016727 TVHUYS	PLACENTAL-LIKE ALKALINE PHOSPHATASE PRECURSOR (EC 3.1.3.1) Homo sapiens (Human).
	AAG41947	protein-tyrosine kinase (EC 2.7.1.112) yes-1 - human AF304164 NID: - Homo sapiens
	2TGD	trypsin (EC 3.4.21.4) precursor (diisopropylphosphorylated) - bovine
	INTP	trypsin (EC 3.4.21.4) (isopropylphosphorylated) - bovine
	YES AVISY	Tyrosine-protein kinase transforming protein YES (EC 2.7.1.112) Avian sarcoma virus (str.
	Q9UBR3	INSULIN RECEPTOR SUBSTRATE PROTEIN OF 53 KDA (A SHORTER FORM) Homo sapiens (Human).
	151593	protein-tyrosine kinase (EC 2.7.1.112) yes - Xiphophorus helleri
	A49114	protein-tyrosine kinase (EC 2.7.1.112) fyn - Pacific electric ray
	KPY1_HUMAN	Pyruvate kinase, M1 isozyme (EC 2.7.1.40) (Pyruvate kinase muscle isozyme) (Cytosolic thyr
	<u>137984</u>	keratin 9, type I, cytoskeletal - human
	<b>B26168</b>	ribophorin II precursor - human
	<u>095A08</u>	SRC-LIKE B (FRAGMENT) Lethenteron reissneri.
	TVHULY	protein-tyrosine kinase (EC 2.7.1.112) lyn, splice form A - human
	PAHUI	alkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human
	<u>Q9JJ10</u> Q9DDK6	NEURONAL C-SRC TYROSINE-SPECIFIC PROTEIN KINASE Rattus norvegicus (Rat). SRC-FAMILY TYROSINE KINASE SCK Salmo salar (Atlantic salmon).
	A46506	leukocyte activation antigen M6 - human
	A44861	keratin, 67K type II epidermal - human
	092957	SRC TYROSINE KINASE Rous sarcoma virus.
	095357	PUTATIVE 6 PROTEIN-COUPLED RECEPTOR (CDNA FLJ10899 FIS, CLONE NT2RP5003506) (RETINOIC ACID
	Q9H2K9	CARBOXYPEPTIDASE M Homo sapiens (Human).
	Q9UHR4	INSULIN RECEPTOR TYROSINE KINASE SUBSTRATE Homo sapiens (Human).
	161771	keratin 6f, type II - human
	AAD45866	AF099011 NID: - Homo sapiens
	AAD05191	MUSKTEP2A NID: - Mus musculus
	<u>Q9D2K8</u>	0 DAY NEONATE HEAD CDNA, RIKEN FULL-LENGTH ENRICHED LIBRARY, CLONE:4833436C19, FULL INSERT
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In any search of a large LC-MS/MS dataset

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Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
20	491.66	981.31	980.44	0.87	0	35	1	QMVVCCNK + Carbamidomethyl (C)
16	464.68	927.35	926.41	0.94	0	29	1	FCNLGMR + Oxidation (M); Propionamide (C)
44	604.15	1206.29	1205.65	0.63	0	24	1	EEGDYVLVLK + 3 Methyl ester (DE)
4	787.30	786.29	785.46	0.82	0	23	1	QVLAEVK
2	825.34	824.33	824.51	-0.18	0	22	1	IVVPEIR
6	403.66	805.29	804.41	0.89	0	21	1	MDLSPVK + Oxidation (M)
77	711.73	1421.45	1420.77	0.67	2	20	1	FDAKLMLEKLR + Acetyl (N-term); Oxidation (M)
58	659.17	1316.32	1315.61	0.71	0	20	1	WDPLDFPDNK + Acetyl (N-term); 2 Methyl ester (DE)
19	491.20	980.38	980.54	-0.15	0	20	1	LINSFLMK + Oxidation (M)
24	515.19	1028.36	1028.45	-0.09	0	18	1	GQQDTGPDR + Acetyl (N-term); Methyl ester (DE)
86	761.71	1521.41	1521.82	-0.41	0	18	1	FPAPFPVCLFSIK + Carbamidomethyl (C)
55	642.70	1283.39	1283.70	-0.31	1	18	1	SPKYTHAIVAR + Acetyl (N-term)
167	803.47	3209.84	3209.46	0.38	0	18	1	ERPDGQSYEEQDAAAVGGGGGGSAHQSGANEVK + 5 Methyl ester (DE)
114	894.30	1786.59	1786.97	-0.37	0	17	1	TVDEVVSTGTDIIIVGR + Methyl ester (DE)
14	431.65	861.29	860.37	0.92	0	17	1	EYNYYK + Pyro-glu (N-term E)
113	893.28	1784.54	1784.96	-0.41	2	17	1	KRLENHPSFVFLEK + Acetyl (N-term)
62	670.20	1338.38	1337.57	0.81	1	17	1	KDGSQCCPQCK + Carbamidomethyl (C); Methyl ester (DE);
137	739.52	2215.53	2215.97	-0.43	1	17	1	YWFCYSTKCYYFIMNK + Carbamidomethyl (C)
151	833.57	2497.69	2498.33	-0.64	2	16	1	MTVRIVSNAVNALISGADDNVKR + Acetyl (N-term); Methyl este
2.6	521.20	1040.38	1040.41	-0.03	0	14	1	SIFDCGDDK + Acetyl (N-term)
39	581.66	1161.31	1161.57	-0.26	0	14	1	QQLLEMFEK + Methyl ester (DE); Pyro-glu (N-term Q)
35	554.18	1106.35	1105.55	0.80	0	14	1	ESLCSVLDK + Acetyl (N-term); Carbamidomethyl (C); Meth
136	731.51	2191.52	2190.94	0.58	2	13	1	ECDLTDRADEAERDAEAGR + Acetyl (N-term); 2 Methyl ester
165	788.69	3150.71	3150.41	0.30	1	13	1	EDLRHLCICSVDPPGCTDIDDALHCR + Acetyl (N-term); Carbamid
61	446.81	1337.42	1336.65	0.77	1	13	1	QGRVTWEEYR + Methyl ester (DE)
89	764.71	1527.40	1526.87	0.54	0	13	1	STQLATLVSLSIPR + Acetyl (N-term)
64	453.17	1356.48	1355.73	0.75	2	13	1	SKVEWPAVRER
111	592.87	1775.60	1775.76	-0.16	1	13	1	EKEISDDEAEPEDEK + Methyl ester (DE)
154	855 90	2564 66	2565 17	-0 51	n	13	1	I CYNAI DEEDEMSTAASSSSI EK + Carbamidomethyl (C)
one								

There are always a number of spectra which get poor scores, or even no match at all.



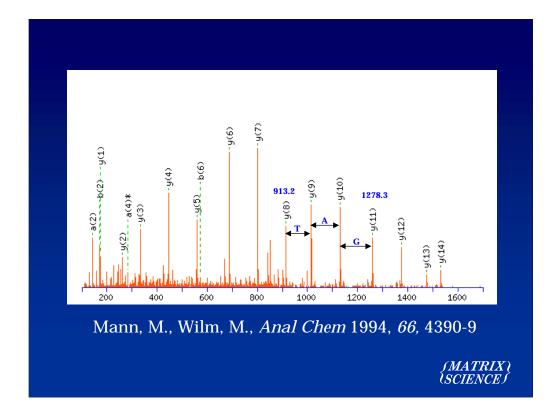
Sometimes, this is because the spectra are empty, or contain little more than noise. However, some of the spectra may contain clear sequence ion ladders at good signal to noise

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Why do the good quality spectra fail to match? Some problems are easily remedied. For example, the precursor charge may have been called incorrectly, or the mass tolerance estimate may be over-optimistic.

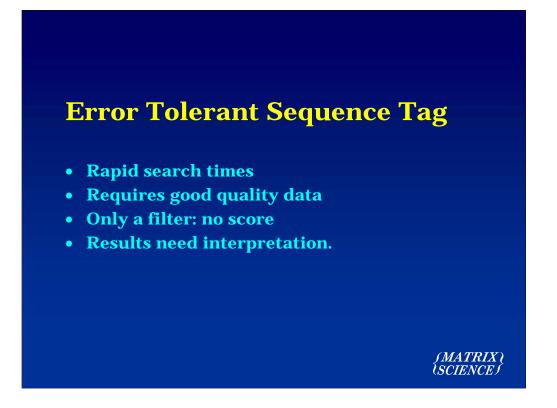
Enzyme non-specificity is very common, and can be addressed with a noenzyme search, but this is very time consuming.

The two other causes of failure to match are more difficult to deal with: Unsuspected modifications and variations in the primary sequence.



The best tool available for finding matches when there are unsuspected modifications or variations in the primary sequence is the error tolerant sequence tag, developed at EMBL. The standard tag combines an interpreted tag with the flanking fragment ion masses, the peptide mass, and the enzyme specificity.

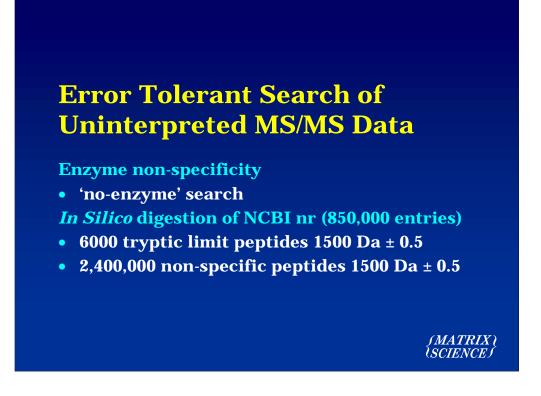
By relaxing one or more of these constraints, the tag can accommodate enzyme non-specificity and / or unexpected mass differences to one side or the other of the tag.



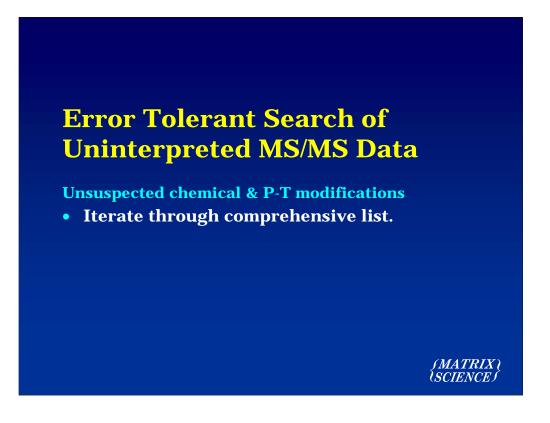
The error tolerant sequence tag is a very fast search, but the data quality has to be high enough to interpret a reliable tag. Also, the error tolerant sequence tag is less specific, and it becomes more likely that multiple hits will be found.

Since there is no score to indicate which hit is the best match, and whether the best match is significant, the user has to reconcile the spectrum to each of the candidate sequences, and decide which match to accept.

To address these limitations, we decided to investigate an error tolerant approach using Mascot to search uninterpreted data



Searching a complete sequence database with no enzyme specificity takes much longer than the same search with (say) tryptic specificity. This is because there are between 100 and 1000 times as many peptides to be tested.



For unsuspected modifications, it would be nice to try all possible mass values at all residue locations. Unfortunately, this doesn't work because all specificity is lost. We have chosen to iterate through a very comprehensive list of modifications

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A         B         C         D         E         F         G         H         J         KL         M         N         O         P           38         PEO Blotin         414.1930         414.14519         PEO Blotin (C)         18         30         4.5         1         1         3         1         3         1         3         1         3         1         3         1         3         1         3         1         3         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1 <th>D 🚅 🖬 🚑 🖪 🖤 🐰 🖻 🛍 🝼 🕬 -</th> <th>ci + 🚷 Σ</th> <th>f≈ 21 31 10 ?</th> <th>Arial 🔹</th> <th>10 • B</th> <th>ΙU</th> <th>E 8</th> <th>8.3</th> <th>•</th> <th>•</th> <th>田.</th> <th>- 🕭</th> <th>- A</th> <th></th> <th></th>	D 🚅 🖬 🚑 🖪 🖤 🐰 🖻 🛍 🝼 🕬 -	ci + 🚷 Σ	f≈ 21 31 10 ?	Arial 🔹	10 • B	ΙU	E 8	8.3	•	•	田.	- 🕭	- A		
Name         Mano         Average         Stort name         Hidden         Notes         C         H         No         P         Stort           39         PEOBloin         414.1970         414.510         PEOBloin         1         1         4         5         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         3         1         1         1         3         1 </th <th>A2 = Acetylation</th> <th>00</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>-</th> <th>_</th> <th>_</th> <th>_</th> <th></th>	A2 = Acetylation	00									-	_	_	_	
38       PEO Bloin (2)															
39       Phosphorylation       79       99       90       97       Phosphorylation       1       3       1       1         41       Phosphorylation       81       93       Phosphorylation       1       3       1       1       3       1         42       Phosphorylation       10       3       1       3       1       1       3       1         43       Porsphorylation with multial loss of HSPO4       150007       16007       15017       17070 C       Propalphol(1)       1       3       1       1         43       Acylamide adduct (propionamide)       71.03712       171070 C       Propalphol(1)       1100712       119123 K       Prydyl(1)       1       1       1       1       1         44       Pyroglu form E       1000712       119123 K       Prydyl(1)       1 <td></td> <td></td> <td></td> <td></td> <td>Hidden</td> <td>Notes</td> <td></td> <td></td> <td></td> <td></td> <td>S</td> <td>Na</td> <td>F</td> <td>S</td> <td></td>					Hidden	Notes					S	Na	F	S	
40       Phosphorylation Neutral loss       97 99500       97 9950 Neutraluss       Phosphorylation       3       4       1         42       Phosphorylation       79 9950       97 9950 Neutraluss       Phosphorylation       3       1       3       1         42       Phosphorylation with neutral loss of HDP04       160 1057       118 015 S,T       Phosphorylation       2       2       1							18		4			1			
41       Phosphorylation       79 9603       79 9800 'V       Phosphorylation       1       3       1       1         42       Phosphorylation with neutral loss of the Photope (')       10       3       6       1       1       1       3       1         43       Acrylanida adduct (propionamide)       71 03712       171 037 0       Propionamide (C)       3       6       1       1       1         44       Prodyl       119 03712       119 123 NEm       Projul (Nem O)       7       6       1       1       1       1         45       Prodyl from E       110 03712       119 123 NEm       Projul (Nem O)       3       1       1       1       1         46       Prodyl from D       17 005 ResidueNEm Prog (Nem O)       6       9       1       2       1       1         47       Prodyl from D       17 005 ResidueNEm Prog (Nem O)       6       9       2       1									_			-		-8	
42       Phosphorylation with neutral loss of H9P04       -18.01057       11.8015 S,T       PhosphorNL(57)       11.01         44       Pyrrdyl       119.027/2       171.072       Pyrrdyl       Nidden       7       6       1       1         44       Pyrrdyl       119.027/2       171.072       Pyrrdyl (Nem       Nidden       7       6       1       1         46       Pyrrdyl       119.027/2       171.9123 K       Pyrrdyl (Nem       Nidden       7       6       1       1         47       Pyrrdyl from E       -18.01057       18.016 ResiduesNtem Pyrogl (Nem E)       -2       1       1         48       SMA       127.06333       127.143 Ntem       SMA (No       6       9       1       2         50       Sodium adduct       21.99194       21.982 Ctem       Sodiated (Ctemm)       -1       1       1         51       Sodium adduct       21.99194       21.982 Ctem       Sodiated (Ctemm)       -1       1       1         52       Sorg/dytethylation       105.0576       105.198 C       Symdylater (Mem       1       2       1         52       Sorg/dytethylation       14.01556       14.027 CkR/H,D,E,N, Methylation (em) hidden       1												-		-8	
42       Acylamide adduct (propionamide)       71.03712       71.0379 C       Propionamide (C)       3       6       1       1       1         44       Prindyl       119.03712       119.123 Nerm       Prindyl (N)       hidden       7       6       1       1       1         45       Prindyl       119.03712       119.123 Nerm       Prindyl (N)       hidden       7       6       1       1       1         46       Prindyl from E       -18.0105 ResidueNterm Proglu (Nerm D)       -2       -1       1       1         47       Prosqlu from O       -17.0035 ResidueNterm Proglu (Nerm O)       -3       -1       1       1         48       SNA       127.0633       127.143 Nerm       SNA (N)       6       9       1       2       1         49       SNA       127.0633       127.143 Nerm       SNA (N)       7       7       1       1       1         51       Solum adduct       21.98198       11980 DE       Sodured (C+erm)       -1       1       1       1         51       Solum adduct       21.98196       13.999 M       Subhora       1       2       1       1         52       Staphora					12.01						1	-			
44         Pyridyl         Pyridyl         Nidden         7         6         1         1           46         Pyridyl         119.027/12         119.123 Ntarm         Pyridyl (Nerm)         Nidden         7         6         1         1           46         Pyridyl forn E         -10.01057         -18.015 ResiduesNterm Pyrogle (Nerm E)         -2         1         1           47         Pyrodyl forn E         -12.015 ResiduesNterm Pyrogle (Nerm E)         -3         1         1           48         SMA         127.05333         127.143 Ntarm         SMA (Norm)         6         9         1         2         1           50         Sodium adduct         21.99194         21.902 Cterm         Sodiated (Cterm)         -1         1         1           52         Sorg/dytethylation         0156785         165.190 C         Sorg/mdytethylation         1         2         1           52         Sorg/dytethylation         14.0565         14.027 C K/R H,D.E/N, Merthylation (em)         1         1         1           53         Solghorn         2.99402         1.9960 N Sulgherm         1         2         1           54         Ctrulination         1.401656         14.027 C K/R H,D.E/N, Merhylat					hidden						_	_		- 88	
45         Prind/ Prind/ Provalu from E         119 027/12         119 123 Narm         Prind/ Pr					12.11						_			-8	
4E         Piroglu fon E         -10.01027         -11.0105         -2         -1           4E         Piroglu fon D         -17.0256         -17.030         -2         -1         -1           4E         SMA         -17.0256         -17.030         SMA (P)         -6         9         1         2           4E         SMA         127.0633         127.143 km         SMA (P)         -6         9         1         2           4B         SMA         127.06333         127.143 km         SMA (P)         -6         9         1         2           5D         Sodium adduct         21.99194         21.922.0 L         Sodiatel (C-term)         -1         1         1           5D         Sodium adduct         21.99194         21.922.0 L         Sodiatel (DE)         -1         1         1           5D         Sodium adduct         21.99194         21.922.0 L         Sodiatel (DE)         -1         1         1           5D         Sodium adduct         0.99628         0.9950 M         Sulphone         1         2         1           5D         Sodium adduct         1.0956         14.027.0 C.K.R.H.D.F.M. Methylation (rem) hidden         1         2         1												-		-8	
47       Pix-oglu (hm O)       -3       -1       -1       -1         48       SMA       127 06333       127 143 Nterm       SMA ( $\gamma$ )       66       9       1       2         49       SMA       127 06333       127 143 Nterm       SMA ( $\gamma$ )       66       9       1       2       4         49       SMA       127 06333       127 143 Nterm       SMA ( $\gamma$ )       66       9       1       2       1         51       Sodium adduct       21 98194       21 9820 Clem       Sodiated (DE)       -1       1       1       1         51       Sodium adduct       21 98194       21 9820 Clem       Sodiated (DE)       -7       7       1       1       1         53       Staphone       13 9850 Sl       Staphone ( $hidden$ 1       2       1       1       1         55       Staphone       14 007 CK PLA DE, M, Mtehnylaion (res)       hidden       1       2       1					nidden						_	-		- 88	
48         SMA         127 143 K         SMA (n)         6         9         1         2           48         SMA         127 06333         127 143 K         SMA (N+m)         6         9         1         2           50         Sodium adduct         21 99194         21 922 Clarm         Sodiuted (0-term)         -1         1         1           52         Sodium adduct         21 99194         21 922 DL         Sodiated (0-term)         -1         1         1           52         Sorgindytethylation         156 05765         105 139 C         S-ypridytethylation         1         2         1           52         Sorgindytethylation         13 0560576         14027 C /L, R, PL, D, E, M, Methylation (em)         1         2         1           54         Chrulination         14 01256         14 027 C /L, R, PL, D, E, M, Methylation (em)         1         2         1           56         Methylation         15 09 C /L, R, PL, D, E, M, Methylation (em)         1         2         1         1           56         Methylation         16 020 54 C /L, R, PL, D, E, M, Methylation (em)         1         2         1         1           56         Methylation         10 026 54 C /L, R, PL, D, E, M, Methylation (em)								-2		-1				- 88	
49         SNA         127 (12) Nam         SNA (h Lem)         6         9         1         2         1           50 Sodium adduct         21 99194         21 992 Crem         Sodiated (CLem)         -1         1         1         1           51 Sodium adduct         21 99194         21 992 D.C         Sodiated (CLEM)         -1         1         1         1           51 Sodium adduct         21 99194         21 992 D.C         Sodiated (CLEM)         -1         1         1         1           52 Sorphighthylation         105 05768         105 1990 M         Sulphone (M)         -1         1							6			2	-	-		- 22	
S00         Sodium adduct         21 98194         21 982 (2mm         Sodium adduct         -1         1           S01         Sodium adduct         21 99194         21 982 (D.E. Sodiated (D.Em)         -1         1           S02         Sodium adduct         105 139 C. D.E. Sodiated (D.Em)         -1         1         1           S02         Sodium adduct         105 139 C. S. Sprindylethyl (O)         7         7         1         2           S02         Sodium adduct         0.99602         O.965 R. Chrulination         hidden         1         2         2           S64         Mithylation         14.01556         14.027 C.K.R.H.D.E.M. Methylation (em) hidden         1         2         2           S66         Mithylation         15.09 C.K.R.H.D.E.M., Methylation (res) hidden         1         2         2           S67         Hydroxylation         16.09 C.K.R.H.D.E.M., Methylation (res) hidden         2         4         2           S67         Hydroxylation         20.05 C.K.R.H.D.E.M., Methylation (res) hidden         2         4         2           S61         Mithylation         20.056 C.K.R.H.D.E.M., Methylation (res) hidden         3         6         2         2           S61         Mithylation         10.056											_	-		- 88	
Sind maddact         21 99194         21 992 D,E         Sodiated (DE)         -1         1         1           Sin younderthylation         105 0.7576         105 139 C         Syndyderthylation         7         1         2         2           Sin younderthylation         0.99083         31 999 M         Sulphone (M)         7         1         2         2           Sin younderthylation         0.99083         31 999 M         Sulphone (M)         1         2         2           Sin younderthylation         14 01565         14 027 C K (R H, D, E, M, Mehrylation (ere) hidden         1         2         1         2         2           Sin Mehrylation         16 99422         15 999 P, K, D, M, Hwithylation (ere) hidden         1         2         1         2									- 1	~		1			
Size Sprudytethylation         105.05766         105.190 C         Symphylathyl(C)         7         7         1         2           SJulphone         31.99893         31.999 M         Sulphone (M)         -         1         -         2         2           SUbphone         0.98402         0.9865 R         Chrulination         hidden         1         -         1         1         1         -         -         1         1         1         -         -         1         1         1         -         -         1         1         1         -         -         1         1         1         -         -         1         1         -         -         1         1         -         -         1         1         - </td <td></td> <td>- 88</td> <td></td>														- 88	
33         Sulphone         31.9990 M         Sulphone (h)         2         2           43         Sulphone         0.9940 0.9966 P         Chrulination         hidden         1         1         1         1           55         Methylation         14.01566 14.027 CK PH JD, EN, Methylation (eers)         hidden         1         2         1         1           56         Methylation         15.9949 PK D, M         Hydroxylation         12         1         1         2         1         1         2         1         1         2         1         2         1         1         2         1         1         1         2         1         1         1         2         1         1         2         1         1         2         1         1         2         1         1         2         1         1         2         1         1         2         1         1         2         1         1         2         1         1         2         1         1         2         2         1         1         1         1         1         1         1         1         1         1         1         1         1         1							7	7	1						
54         Chrulination         0.98402         0.98678         Chrulination         idden         -1         1         1           55         Methylation         14.07565         14.027 C,K,R,H,D,E,M, Methylation (res)         hidden         1         2         1           56         Methylation         15.9842         15.999 P,K,D,M         Hydroxylation         1         2         1         1           56         Methylation         20.0130         28.054 C,K,R,H,D,E,M, Methylation (res)         hidden         1         2         1           56         Methylation         28.0130         28.054 C,K,R,H,D,E,M, Methylation (res)         hidden         2         4         1           56         Methylation         28.0310         28.054 C,K,R,H,D,E,M, tethylation (res)         hidden         2         4         1           50         interMethylation         42.0465         42.000 C,K,R,H,D,E,M, tethylation (res)         hidden         3         6         1         2         1           50         interMethylation         42.010 D,E         Garama-actioxylation hidden         1         2         1         1           50         Betra-methylthylation         10         0         5         3         1 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>2</td> <td></td> <td></td> <td></td> <td></td> <td></td>										2					
Bit         Mithylation         14.07566         14.027 Residues/Narm Methylation (mm)         hidden         1         2            SPL Hydroxylation         15.9942         15.999 PK, D,N         Hydroxylation         1         2         1         1           SB di-Methylation         28.03130         28.054 C K, R.H.D,R. M, eMethylation (res)         hidden         2         4         1         1           SB di-Methylation         28.03130         28.054 C K, R.H.D, R.M., Methylation (res)         hidden         2         4         1         1           SD it-Methylation         42.04805         42.080 C K, R.H.D, R.M., thethylation (res)         hidden         3         6         1         1         2         1           SD it-Methylation         42.04805         42.080 C K, R.H.D, R.M., thethylation (res)         hidden         3         6         1         2         1           SD attern-methylholation         42.04805         42.010 D.E         Garama-carboxylation hidden         1         2         1           SD attern-methylholation         43.9895         44.010 D.E         Sublation         1         2         1         1           SD attern-methylholation         43.9895         45.002         Sublation         1 <td></td> <td>0.98402</td> <td>0.985 R</td> <td>Citrullination</td> <td>hidden</td> <td></td> <td></td> <td>-1</td> <td>-1</td> <td>1</td> <td></td> <td></td> <td></td> <td></td> <td></td>		0.98402	0.985 R	Citrullination	hidden			-1	-1	1					
327         Hydroxylation         15.99422         15.999 P,K.D.N.         Hydroxylation         1         1         1           930         di-Methylation         20.0130         28.054 C,K.P.H.D.E.N. M-Methylation (rep.)         hidden         2         4         1         1         2         1         2         1	55 Methylation	14.01565	14.027 C.K.R.H.D.E.N	Methylation (res)	hidden		1	2							
Bit         Operating and the state of					hidden		1	2							
Sgl di-Methylation         20 03130         28 054 Residues/Netm di-Methylation (sem)         Indden         21         4         2           00 tri-Methylation         42 0406         42 000 C Residues/Netm tri-Methylation (sem)         Nidden         3         6         -					hidden					1					
BDI tri-Methylation         42 04895         42 0080 C, K, P, H, D, EN, tri-Methylation (resp.)         Iniden         3         6         5           Bit tri-Methylation         42 04895         42 008 0 C, K, P, H, D, EN, tri-Methylation (resp.)         Iniden         3         6         5           Bit tri-Methylation         43 98983         44 010 D, E         Gamma-carboxylation         1         2         1           Bit amethylholation         45 98983         44 010 D, E         Gamma-carboxylation Inden         1         2         1           Bit amethylholation         45 98983         44 010 D, E         Gamma-carboxylation Inden         1         3         1           Bit amethylholation         73 98962         90 066 Y         Sublation         Inden         1         3         1           Bit amethylholation         152 05283         73 980 H, C, D         Phosphorylation Inden         6         1         3         1           Bit C, Mannosylation         152 05283         12 42 W, T K         C Mannosylation Inden         6         10         5           Bit G, Sylation         152 05283         12 42 W, T K         C Mannosylation Inden         6         10         5           Bit G, Sylation         150 05283         12 42 W, T															
Bit Interfundation         42.08895         42.0897         02.0807         04.0819 <td></td>															
B2C         Samous-carboxylation         43 98983         44 010 D_E         Gamma-carboxylation hidden         1         2         1           B2D         Bata-methythiolation         45 9872         46 B07         DB eta-methythiolation hidden         1         2         1           B4         Suffation         73 95662         80 058 Y         Suffation         1         2         1           B5         Phosphorylation         73 95633         79 960 H_C P         Phosphorylation         10         3         1           B6         CMannosylation         162 05263         162 142 W         C-Mannosylation         10         5           B6         CMannosylation         162 05263         162 142 W         C-Mannosylation         61 10         5           B6         Openion         162 05283         162 142 N/L Mem Glycation (term) hidden         61 10         5           B6         Openion         162 05283         162 142 N/L Mem Glycation (term) hidden         61 10         5           B9         Upoyl         188 032/E         Lipoyl         hidden         81 12         1         2           D9 OciGNec         203 07938         233 194 S,TN         OciGNexa         81 13         5         5															
Big Betwenthythiolation         45.98772         46.087 D         Betwenthythiolation hidden         1         2         1           Big Betwenthythiolation         79.96831         79.96031         Sulfation         hidden         1         3         1           Big Dephytiktion         79.96831         79.96031         Phosphorytation         1         3         1           Big Dephytiktion         162.05281         162.142 W         CManosytation         1610         5           Big Ockwanosytation         162.05281         162.142 W         CManosytation         610         5           Big Ockwanosytation         162.05281         162.142 N/T.K         Glycation (Perro)         hidden         610         5           Big Ockwanosytation         162.05281         162.142 N/T.K         Glycation (Perro)         hidden         610         5           Big Ockwanosytation         162.05281         162.142 N/T.K         Glycation (Perro)         hidden         610         5           Big Upoyl         188.032/K         Upoyl         hidden         610         5           Di OciciNec         203.079396         233.914 S,TN         OciciNechaa         hidden         8112         1         2								6							
GHI Suttion         79 96862         80 056) Y         Sulfation         hidden         3         1           GF PhosphoryNation         79 9682         980 H C.D         PhosphoryNation         1         3         1           96 C-Mannosytation         162 05283         162 142 W         C-Mannosytation         6         10         5           96 C-Mannosytation         162 05283         162 142 W         C-Mannosytation         6         10         5           96 G-Mannosytation         162 05283         162 142 N,T-K         Glycation (terr)         hidden         6         10         5           96 G-Station         162 05283         162 142 N,T-K         Glycation (terr)         hidden         6         10         5           96 G-Station         162 05283         162 142 N,T-K         Glycation (terr)         hidden         6         10         5           97 G-Station         160 05286         162 142 N,T-K         Glycation (terr)         10         5           98 Upoyl         188 032/6         149 07         Lipoyl         hidden         8         12         1         2           97 O-GicNac         2030 07398         233 91 N,T         O-GicNac         8         15         5									_	2					
BEG         Phosphorylation         179 96633         79 9800 H.C.D         Phosphorylation         1 diden         1         3 1           BEG         CMannosylation         162 02283         162 142 W         CMannosylation         1610         5           BEG         Classical Science         124 22 W         CMannosylation         610         5           BEG         Science         124 22 NT/K         Glycation (Perro)         hidden         610         5           BEG         Science         124 22 NT/K         Glycation (Perro)         hidden         610         5           BEG         Science         124 22 NT/K         Glycation (Perro)         hidden         610         5           BEG         Science         124 23 NT/K         Glycation (Perro)         hidden         610         5           BEG         Science         120 37936         183 302 K         Lipoyl         hidden         611         2           D0         OciNec         203 07936         233 394 57.N         OcyCeNac         hidden         811         5							1	2		0					
BB         C-Mannosylation         162 05281         162 142 W         C-Mannosylation         Inidian         61 10         5           67         Olycation         162 05283         162 142 N,TK         Olycation (res)         Inidian         61 10         5           68         Olycation         162 05283         162 142 N,TK         Olycation (res)         Inidian         61 10         5           89         Upoyl         188 032/6         183 902 K         Lipoyl         Inidian         61 10         5           70         OciSiCNac         2030 073908         203 914 S,TN         OciSiCNac         81 13         1         5												1			
BFC         Skycation         162.05283         162.142         N,T/K         Glycation (res)         hidden         6         10         5           BFC         Skycation         162.05283         162.142         N/TM         Glycation (res)         hidden         6         10         5           BFC         Skycation         162.05283         162.142         N/TM         Glycation (res)         hidden         6         10         5           BFC         Uppyl         Node         8         12         1         2         1         2           DFD         OcideNace         203.073996         233.918         5.71N         Oc/EcNac         11.054         1         5							0		-		1	-			
BB         Glycation         162.05283         162.142 Nerm         Glycation (term)         hidden         61         0         1           69         Lipoyi         186.03296         188.032 K         Lipoyi         hidden         61         2         1         2           70         O-GicNacc         203.07396         203.194 S,T.N         O-GicNac         Hidden         61         1         1         5									-		-	-			
E9 Lipoyl 188.03296 188.302 K Lipoyl hidden 8 12 1 2 70 0-GlcNac 203.07938 203.194 (S,T,N O-GlcNac hidden 8 13 1 5 5555 5555 5555 5555 5555 5555									-			-			
70 O-GicNac 203.07938 203.194 S.T.N O-GicNac hidden 8 13 1 5									-			2			
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204-10761 204-305 C Partneyfallon 10524 72 Myristovlation 2010/19837 2113-359 K Myristovlation (res) hidden 14 26 1										1					
12 minisoprauon (± 2000 autor) 210.333 K minisoprauon (± 2000 autor) (± 2000 aut						-			-			-			

The longer the list, the better. Unfortunately, there is no standard database of modifications that is suitable for MS use, so we had to compile this list ourselves

## Error Tolerant Search of Uninterpreted MS/MS Data

Peptide sequence not in the database

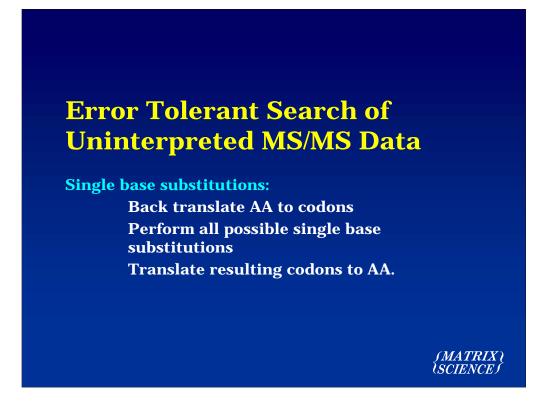
- single base substitutions Yes
- single base insertions & deletions No (frame shift)
- multiple base substitutions, insertions & deletions No (*de novo*).

Virtually all of the protein database entries come from translations of DNA or RNA. Hence, for variations in the primary sequence, it is essential to consider changes at the NA level.

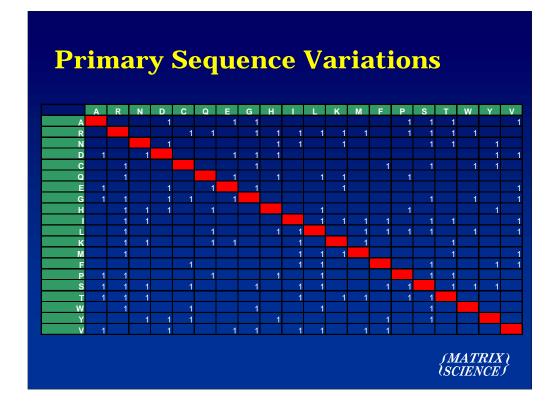
*(MATRIX)* (SCIENCE)

We choose to allow all possible single NA substitutions, but not insertions or deletions, because these give rise to frame shifts.

We also draw the line at multiple variations in a single peptide. These must be handled by de novo sequencing



The procedure to generate a substitution matrix for all possible single base substitutions



This matrix is considerably sparser than all possible residue substitutions

### **Error Tolerant Search of Uninterpreted MS/MS Data**

### Procedure

- Perform standard search
- Select one or more protein hits
- With no enzyme specificity, iterate through extensive list of modifications + substitution matrix.

*(MATRIX)* (SCIENCE)

The error tolerant search is a second pass search

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	in (EC 3.4.2		7 <b>Total sco</b> sor (diisopr error tolera	opylph	losphory	ylated)	- bovi	ne	
Query	Observed	Mr(expt)	Mr(calc)		Miss			Peptide	
	403.13	804.25	804.41	-0.16		65	1	SAASLNSR	
<u>₽ 90</u>	577.17	1152.32	1152.57	-0.25		87	1	SSGTSYPDVLK	
<u>₹ 93</u>	584.67	1167.33	1167.57	-0.25		97	1	VCNYVSWIK + Carbamidomethyl (C)	
✓ <u>100</u>	598.18	1194.34	1194.58	-0.24		(68)	1	SSGTSYPDVLK + Acetyl (N-term)	
✓ <u>173</u>	745.72	1489.43	1489.73	-0.31		78	1	LQGIVSWGSGCAQK + Carbamidomethyl (C)	
✓ <u>195</u>	805.17	1608.32	1608.65	-0.33		79	1	DSCQGDSGGPVVCSGK + 2 Carbamidomethyl (C)	
289	1081.77	2161.52	2162.05	-0.53		138	1	LGEDNINVVEGNEQFISASK	
✓ 290	721.88	2162.60	2162.05	0.56		(87)	1	LGEDNINVVEGNEQFISASK	
✓ 296	1102.80	2203.59	2204.06	-0.47	0	(102)	1	LGEDNINVVEGNEQFISASK + Acetyl (N-term)	
✓ 297	735.54	2203.60	2204.06	-0.46		(46)	1	LGEDNINVVEGNEQFISASK + Acetyl (N-term)	
<u>305</u>	758.55	2272.63	2272.15	0.48	0	29	2	SIVHPSYNSNTLNNDIMLIK	
<u>1TAWA</u> trypsi <u>BAA07516</u>		s: 23276 1.4), chair s: 25408	of peptides: Total score: h & - bovine Total score:	571	-				
1AUJ		s: 23290	Total score:	571	Peptide	es matel	hed: 11		
TRBOTR		s: 23978	Total score:		Peptide	es matcl	hed: 11		
trypsi 1MCUE		1.4) precur s: 23203	sor - bovine Total score:		Pentide	s match	hed: 11		
			n E - bovine						
1TGSZ			Total score:						
tryps: 2TPIZ			sor (with pa Total score:					inhibitor), chain Z - bovine	
								Ile-Val, 2.4 M magnesium sulfate), chain Z - bovine	
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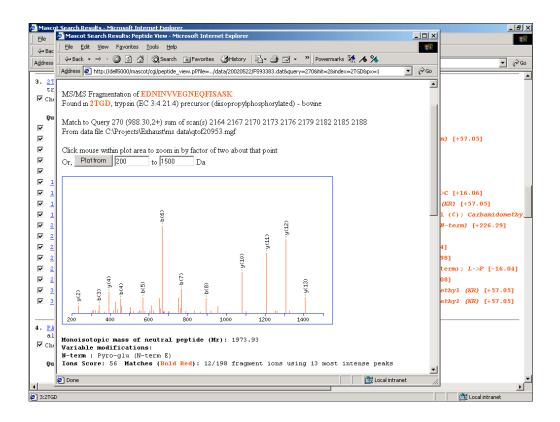
Here we see the matches to trypsin autolysis products from a standard search of an LC-MS/MS dataset from the analysis of a human cell lysate.

Masc	ot Searc	h Results - Mic	rosoft Internet	t Explorer					_ 6 ×
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3. <u>21</u>			<b>fass:</b> 23177						
				sor (diisopr		-			
l♥ Ch	eck to	) include t	his hit in	error toler	ant sea	rcn or	archi	ve repo	rt
Qu	lery	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
•	<u>6</u>	403.13	804.25	804.41	-0.16	0	65	1	SAASLNSR
1	15	431.63	861.24	861.43	-0.19	0	(51)	1	SAASLNSR + Carbamidomethyl (N-term) [+57.05]
•	74	559.15	1116.29	1116.52	-0.23	0	56	1	S IVHP SYNSN
•	90	577.17	1152.32	1152.57	-0.25	0	87	1	SSGTSYPDVLK
~	93	584.67	1167.33	1167.57	-0.25	0	97	1	VCNYVSWIK + Carbamidomethyl (C)
	.00	598.18	1194.34	1194.58	-0.24	0	(68)	1	SSGTSYPDVLK + Acetyl (N-term)
V 1	<mark></mark> ]իոյ	606.19	1210.35	1210.55	-0.20	0	(57)	1	SSGTSYPDVLK + Acetyl (N-term); S->C [+16.06]
V 1	- <u></u>	745.72	1489.43	1489.73	-0.31	0	84	1	LQGIVSWGSGCAQK + Carbamidomethyl (KR) [+57.05]
V 1				hes to query					CQGDSGGPVVCSGK + Carbamidomethyl (C); Carbamidomethy
✓ 2				653 1656 165 s for this p		1665 :	1668 16	071 1674	INVVEGNEQFISASK + Biotinylated (N-term) [+226.29]
2	8	· · · · · · · · · · · · · · · · · · ·		,					FEDNINVVEGNEQFISASK
2	9 Scot 57		Hit 3	Protein 2 TGD	Peptid SSGTSY				EDNINVVEGNEQFISASK + Q->K [+0.04]
2	9 57		3	2 TGD	SSGTST				EDNINVVEGNEQFISASK + L->H [+23.98]
✓ 2	9 44		3	2 TGD					EDNINVVEGNEQFISASK + Acetyl (N-term); L->P [-16.04]
2	9 36 34		3	2 TGD	KTSFTP SSGTSY				EDNINVVEGNEQFISASK + G->V [+42.08]
V 3			Ŭ	8105	GGSGGG		GRGS		EDNINVVEGNEQFISASK + Carbamidomethyl (KR) [+57.05]
V 3	0 28				GGSGGG				EDNINVVEGNEQFISASK + Carbamidomethyl (KR) [+57.05]
	26				GSGGGG GARSMS				
4. PJ	H 17	.5 -0.15			SGYR <u>S</u> G	GGF <u>s</u> so	3S		
al									
🗹 Ch	eck to	) include t	his hit in	error toler	ant sea	rch or	archi	ve repo	rt
		01	Martmark	Mar ( 1 1	B-14	M4 -	C	<b>D</b> 1	Deuteide
-	lery 52	Observed 517.18	Mr(expt) 1032.34	Mr(calc) 1032.56	<b>Delta</b> -0.22	Miss	Score 67	Rank 2	Peptide GSSIFGLAPSK + S->G [-30.03]
	24	517.10	1032.34	1032.56	-0.22	0	107	4	CONTROLATION + S-AG [-SU.US]
] 3:2TG	iD								🕂 Local intranet

And here are the matches from the second pass, error tolerant search. The number of matches has increased from 10 to 17. Only those matches that were as good as or better than the original search are reported.

Query 74 is a simple, non-specific cleavage product

Query 107 shows acetylation at the N-terminus plus a mass increase of 16 at one of the N-term serines. It is not easy to find a reasonable mechanism to account for either an increase of 16 or an increase of 58 (42+16) at the N-term of this peptide

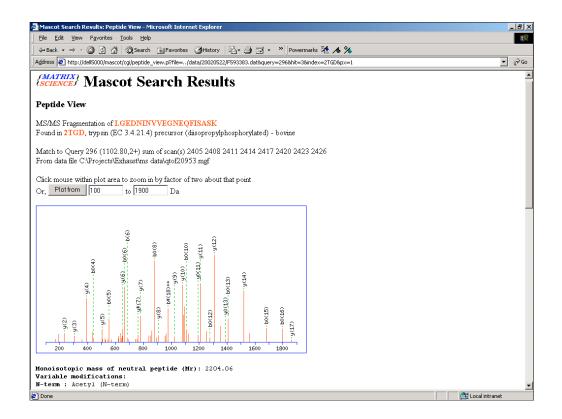


Query 270 is assigned to NINVVEGNEQFISASK with a biotinylated Nterm. However, this sample was not biotinylated, so this assignment cannot be correct. In fact, we get exactly the same mass increment by moving the cleavage point two residues further towards the N-terminus and adding a pyro-glu modification. This is a good illustration of how difficult it is for the report to always give the correct assignment

→ → → → → → → → → → → → → → → → → → →	Mascot Sea	rch Results - Mi	crosoft Interne	t Explorer					
intro://delCool/macco/cglnew_master_results.ghtHem./data/2000522/F9333.dd       Image: Control of the second	<u>Eile E</u> dit	⊻iew F <u>a</u> vorites	<u>T</u> ools <u>H</u> elp						
2750       Hass: 23177       Total score: 482       Peptides matched: 17         trypsin (EC 3.4.21.4)       precursor (disoproylphosphorylated) - bovine         Z (heck to include this ht in error tolerant search or archive report         Query Observed Mr(expt)       Mr(calc)       Delta Miss Score Rank       Peptide         Z (beck to include this ht in error tolerant search or archive report         Query Observed Mr(expt)       Mr(calc)       Delta Miss Score Rank       Peptide         Z (b 403.13       004.25       004.41       -0.16       0       65       1       SAASLINSR         Z (b 403.13       004.25       004.41       -0.16       0       65       1       SAASLINSR         Z (b 50.17       116.52       0.023       56       1       STMPSTNSN       (0)       (0)         Z (100       598.18       1194.34       1194.58       -0.24       0       (69)       1       SSGTSYPDVLK + Acetyl (M-term)       S>-C [+16.06]         Z 102       606.19       1210.35       1210.55       -0.20       0       (57)       1       SGGDSGAVCSGAK + Carbanidomethyl (C)       Carbanidomethyl (RR) [+57.05]         Z 102       606.19       1210.35       1210.55       -0.20       0       57       1       NINVEGREQFIS	⇔Back 🕶 =	) - 🗵 🙆 🕹	🖁 🧟 Search 🛛	😹 Favorites 🛛 🕃	History	à- 🎒	<b>I</b> •	>> Power	marks 🌃 🔏 🏂
Trypsin (EC 3.4.21.4) precursor (diisopropylabsphorylated) - bovine           Check to include this hit in error tolerant search or archive report           Query Observed Mr(expt) Mr(calc) Delta Miss Score Rank Peptide           2         6         403.13         604.25         604.41         -0.16         0         65         1         SAASLNSR           2         15         431.63         661.24         661.43         -0.19         0         (51)         1         SAASLNSR           2         15         431.63         661.24         661.43         -0.25         0         67         1         SSGTSYPDVLK           2         90         577.17         1152.32         1152.57         -0.25         0         97         1         VCRVSYPIK + Carbanidomethyl (C)           2         90         577.17         1152.32         1152.57         -0.22         0         97         1         VCRVSYPIK + Carbanidomethyl (C)           2         100         598.18         1194.34         1194.58         -0.20         0         77         1         SSGTSYPUK + Acetyl (M-term)         <->>         SC         (F1.66]           2         1027         606.19         121.055         -0.20         0         63 <t< th=""><th>ddress 🦉 ht</th><th>tp://dell5000/mas</th><th>cot/cgi/new_maste</th><th>er_results.pl?file=.</th><th>./data/20020</th><th>1522/F593</th><th>3383.dat</th><th></th><th>▼ <i>∂</i></th></t<>	ddress 🦉 ht	tp://dell5000/mas	cot/cgi/new_maste	er_results.pl?file=.	./data/20020	1522/F593	3383.dat		▼ <i>∂</i>
Trypsin (EC 3.4.21.4) precursor (diisopropylabsphorylated) - bovine           Check to include this hit in error tolerant search or archive report           Query Observed Mr(expt) Mr(calc) Delta Miss Score Rank Peptide           2         6         403.13         604.25         604.41         -0.16         0         65         1         SAASLNSR           2         15         431.63         661.24         661.43         -0.19         0         (51)         1         SAASLNSR           2         15         431.63         661.24         661.43         -0.25         0         67         1         SSGTSYPDVLK           2         90         577.17         1152.32         1152.57         -0.25         0         97         1         VCRVSYPIK + Carbanidomethyl (C)           2         90         577.17         1152.32         1152.57         -0.22         0         97         1         VCRVSYPIK + Carbanidomethyl (C)           2         100         598.18         1194.34         1194.58         -0.20         0         77         1         SSGTSYPUK + Acetyl (M-term)         <->>         SC         (F1.66]           2         1027         606.19         121.055         -0.20         0         63 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>									
Check to include this hit in error tolerant search or archive report         Query       Observed       Mr(expt)       Mr(calc)       Delta       Miss Score       Rank       Peptide         2       6       403.13       804.25       804.41       -0.16       0       65       1       SAASLNSR         2       15       431.63       861.24       861.43       -0.19       0       (51)       1       SAASLNSR + Carbanidomethyl (N-term) [+57.05]         2       92       577.17       1152.32       116.52       -0.25       0       67       1       SSOTSYPDVLK         2       92       584.67       1167.33       1167.57       -0.25       0       97       1       VCNYSWIK + Carbanidomethyl (C)         2       92       581.8       194.34       1194.58       -0.24       0       (66)       1       SSOTSYPDVLK + Acetyl (M-term); S->C [+16.06]         2       107       606.19       1210.35       1210.55       -0.20       0       (57)       1       SSOTSYPDVLK + Acetyl (M-term); S->C [+16.06]         2       712       74.72       1489.43       149.73       -0.31       0       79       1       DSCQOBSCQLVCSGK + Carbanidomethyl (C); Carbanidomethyl (C); Carbanidomethyl (R); [+57.05]	<ol> <li><u>2TGD</u></li> <li>trungi</li> </ol>								
Outry         Observed         Mr(expt)         Mr(calc)         Delta         Miss Score         Rank         Peptide           2         6         403.13         804.25         804.41         -0.16         0         65         1         SAASLNSR           2         15         431.63         861.24         861.43         -0.19         0         (51)         1         SAASLNSR           2         15         5116.29         1116.52         -0.23         0         56         1         SUMPSYNSH           2         90         577.17         1152.32         1152.57         -0.25         0         97         1         VCHYVSWIK + Carbanidomethyl (C)           2         90         598.18         194.34         1194.58         -0.24         0         (66)         SSUTSYPDVLK + Acetyl (M-term); S->C [+16.06]           2         107         606.19         1210.35         1210.55         -0.20         0         (57)         1         SSUTSYPDVLK + Acetyl (M-term); S->C [+16.06]           2         107         606.19         1210.35         1210.55         -0.20         0         57         1         MSUGPSGEVCAGK + Carbanidomethyl (C); Carbanidomethyl (C); Carbanidomethyl (SR) [+57.05] <t< td=""><td></td><td></td><td></td><td></td><td></td><td>-</td><td></td><td></td><td></td></t<>						-			
2       6       403.13       804.25       804.41       -0.16       0       65       1       SAASLNSR         2       15       431.63       861.24       861.43       -0.19       0       (51)       1       SAASLNSR + Carbanidomethyl (N-term) [+57.05]         2       74       559.15       1116.29       1116.52       -0.23       0       56       1       STUBESTNEN         2       90       577.17       1152.32       1152.57       -0.25       0       87       1       SSGTSYPDVLK         2       90       584.67       1167.33       1167.57       -0.25       0       97       1       VCHYVSWIK + Carbanidomethyl (C)         2       100       598.18       1194.34       1194.58       -0.24       0       (68)       1       SSGTSYPDVLK + Acetyl (N-term); S->C [+16.06]         2       107       606.19       1210.33       120.55       -0.20       0       (57)       1       SSGTSYPDVLK + Acetyl (N-term); S->C [+16.06]         2       107       606.39       1210.33       120.55       -0.20       0       (57)       1       NIVVEGREQFISASK + Acetyl (N-term); S->C [+16.06]       1         2       220       988.30       1974.58<									
2       15       431.63       861.24       861.43       -0.19       0       (51)       1       SAASLNSR + Carbanidomethyl (N-term) [+57.05]         2       74       559.15       1116.29       1116.52       -0.23       0       56       1       SIVHPSYNSN         2       90       577.17       1152.32       1152.57       -0.25       0       97       1       VCHYSWIK + Carbanidomethyl (C)         2       90       581.8       1194.38       -0.24       0       (68)       SSGTSYPDVLK + Acetyl (N-term)         2       100       598.18       1194.35       -0.24       0       (57)       1       SSGTSYPDVLK + Acetyl (N-term)         2       107       606.19       1210.35       1210.55       -0.20       0       (57)       1       SSGTSYPDVLK + Acetyl (N-term)       \$->C [+16.06]         2       107       606.19       1210.35       120.55       -0.30       0       57       1       NINVVEGREQFISASK + Carbanidomethyl (C)	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
<ul> <li>Z 74 559.15 1116.29 1116.52 -0.23 0 56 1 STVHPSINSN</li> <li>Z 90 577.17 1152.32 1152.57 -0.25 0 87 1 SSGTSYPVLK</li> <li>Z 93 584.67 1167.33 1167.57 -0.25 0 97 1 VCHYSWIK + Carbamidomethyl (C)</li> <li>Z 100 598.18 1194.34 1194.58 -0.24 0 (68) 1 SSGTSYPVLK + Acetyl (N-term)</li> <li>Z 107 606.19 1210.35 1210.55 -0.20 0 (57) 1 SSGTSYPVLK + Acetyl (N-term); S-&gt;C [+16.06]</li> <li>Z 173 745.72 1489.43 1489.73 -0.31 0 84 1 LQ0IVSWGSCAQK + Carbamidomethyl (C); Carbamidomethyl (C)</li></ul>	✓ <u>6</u>	403.13	804.25	804.41	-0.16	0	65	1	SAASLNSR
2       90       577.17       1152.32       1152.57       -0.25       0       87       1       SSGTSYPPVLK         2       93       564.67       1167.33       1167.57       -0.25       0       97       1       VCHYVSWIK + Carbanidomethyl (C)         2       100       598.18       1194.34       1194.58       -0.24       0       (68)       1       SSGTSYPVLK + Acetyl (N-term)         2       107       606.19       1210.35       1210.55       -0.20       0       (57)       1       SSGTSYPVLK + Acetyl (N-term)         2       107       606.19       1210.35       1210.55       -0.31       0       64       1       L0GTVSW6SCAQK + Carbanidomethyl (C):       Carbanidom	✓ <u>15</u>	431.63	861.24	861.43	-0.19	0	(51)	1	SAASLNSR + Carbamidomethyl (N-term) [+57.05]
2       92       584.67       1167.33       1167.57       -0.25       0       97       1       VCMYVSWIK + Carbamidomethyl (C)         2       100       598.18       1194.34       1194.58       -0.24       0       (68)       1       SSGTSYPVLK + Acetyl (N-term)         2       107       606.19       1210.35       1210.55       -0.20       0       (37)       1       SSGTSYPVLK + Acetyl (N-term); S ->C [+16.06]         2       107       606.19       1210.35       1210.55       -0.20       0       (37)       1       SSGTSYPVLK + Acetyl (N-term); S ->C [+16.06]         2       107       606.19       1210.35       1210.55       -0.20       0       (37)       1       SSGTSYPVLK + Acetyl (N-term); S ->C [+16.06]         2       1107       606.30       1971.55       0.63       0       71       NINVVEGHEQFISASK + Acetyl (N-term) [+226.29]         2       220       988.30       1974.58       1973.95       0.63       0       57       1       NINVVEGHEQFISASK + 0->K [+0.04]         2       220       721.88       2162.00       2.52       0       92)       1       GEDNINVVEGHEQFISASK + 0->K [+0.04]         2       292       729.53       2185.61	✓ <u>74</u>	559.15	1116.29	1116.52	-0.23	0	56	1	SIVHPSYNSN
7       100       598.18       1194.34       1194.58       -0.24       0       (68)       1       SSGTSYPDVLK + Acetyl (N-term)         7       1007       606.19       1210.33       1210.55       -0.20       0       (57)       1       SSGTSYPDVLK + Acetyl (N-term);       S>C [+16.06]         7       745.72       1489.43       1489.73       -0.31       0       84       1       LQGUSWGSGCAQK + Carbanidomethyl (XX) [+57.05]         7       195       805.17       1608.65       -0.33       0       79       1       DSGGDSGGPVCSGK + Carbanidomethyl (C); Carbanidomethyl (C)	✓ <u>90</u>	577.17	1152.32	1152.57	-0.25	0	87	1	SSGTSYPDVLK
7       606.19       1210.35       1210.55       -0.20       0       (57)       1       SSGTSYPPVLK + Acctyl (N-term); 5->C [+16.06]         2       173       745.72       1489.43       1489.73       -0.31       0       84       1       LQGTVSW6SGCAQK + Carbanidomethyl (KR) [+57.05]         2       195       805.17       1608.52       1608.65       -0.33       0       79       1       DSCGDSSGEVVCSGK + Carbanidomethyl (C); Carbanidometh         2       220       988.30       1974.58       1973.95       0.63       0       57       1       NINVVEGHEQFISASK + Anotyl acd (N-tern) [+226.29]         2       289       1081.77       2161.52       2162.05       -0.53       0       138       1       LGEDNINVVEGHEQFISASK + 2->K [+0.04]         2       290       721.88       2162.60       2162.09       0.52       0       (92)       1       LGEDNINVVEGHEQFISASK + L->H [+2.08]         2       294       1094.81       2187.61       2186.02       -0.44       0       (92)       1       LGEDNINVVEGHEQFISASK + L->H [+42.08]         2       294       1094.81       2187.61       2186.03       -0.42       0       (105)       1       LGEDNINVVEGHEQFISASK + Acctyl (N-term); L->P [-16.04] <td>✓ <u>93</u></td> <td>584.67</td> <td>1167.33</td> <td>1167.57</td> <td>-0.25</td> <td>0</td> <td>97</td> <td>1</td> <td>VCNYVSWIK + Carbamidomethyl (C)</td>	✓ <u>93</u>	584.67	1167.33	1167.57	-0.25	0	97	1	VCNYVSWIK + Carbamidomethyl (C)
7       173       745.72       1489.43       1489.73       -0.31       0       84       1       LQGIVSW6S6CAQK + Carbanidomethyl (KR) [+57.05]         2       195       805.17       1608.32       1608.65       -0.33       0       79       1       DSCQ6DS6GPVVCSGK + Carbanidomethyl (C); Carbanidomethyl         2       270       988.30       1974.58       1973.95       0.63       0       57       1       NINVVECMEQFISASK + Eiotinylated (N-term) [+226.29]         2       290       1081.77       2161.52       2162.05       -0.53       0       138       1       LGEDNINVVEGNEQFISASK + Eiotinylated (N-term) [+226.29]         2       290       721.88       2162.60       2162.09       0.52       0       (92)       1       LGEDNINVVEGNEQFISASK + Q->K [+0.04]         2       291       721.88       2162.60       2162.09       0.52       0       (92)       1       LGEDNINVVEGNEQFISASK + L->H [+23.98]         2       292       721.88       2185.08       2186.02       -0.44       0       (92)       1       LGEDNINVVEGNEQFISASK + Acetyl (N-term) ; L->P [-16.04]         2       294       1094.81       2187.61       2186.03       -0.42       0       (102)       1       LGEDNINVVEGNEQF	✓ <u>100</u>	598.18	1194.34	1194.58	-0.24	0	(68)	1	SSGTSYPDVLK + Acetyl (N-term)
7       195       805.17       1608.62       -0.33       0       79       1       DSCQGDSGGPVVCSGK + Carbamidomethyl (C); Carbamidomethyl	✓ <u>107</u>	606.19	1210.35	1210.55	-0.20	0	(57)	1	SSGTSYPDVLK + Acetyl (N-term); S->C [+16.06]
7       270       988.30       1974.58       1973.95       0.63       0       57       1       NINVVEGHEQFISASK + Eiotinylated (N-term) [+226.29]         2       289       1081.77       2161.52       2162.05       -0.53       0       138       1       LGEDNINVVEGHEQFISASK         2       290       721.88       2162.60       2162.09       0.52       0       (92)       1       LGEDNINVVEGHEQFISASK + 0>K [+0.04]         2       293       729.53       2185.58       2186.02       -0.44       0       (92)       1       LGEDNINVVEGHEQFISASK + L>H [+23.98]         2       294       1094.81       2187.61       2188.03       -0.42       0       (92)       1       LGEDNINVVEGHEQFISASK + Ac->H [+42.08]         2       294       1094.81       2187.61       2188.03       -0.42       0       (92)       1       LGEDNINVVEGHEQFISASK + Acetyl (N-term); L->P [-16.04]         2       296       1102.80       2203.59       2204.10       -0.50       0       (102)       1       LGEDNINVVEGHEQFISASK + Acetyl (N-term); L->P [-16.04]         2       300       1110.30       2218.58       2219.07       -0.49       0       (105)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (KR) [+57.0	✓ <u>173</u>	745.72	1489.43	1489.73	-0.31	0	84	1	LQGIVSWGSGCAQK + Carbamidomethyl (KR) [+57.05]
7       289       1081.77       2161.52       2162.05       -0.53       0       138       1       LGEDNINVVEGHEQFISASK         7       290       721.88       2162.60       2162.09       0.52       0       (92)       1       LGEDNINVVEGHEQFISASK         7       293       729.53       2185.60       2166.02       -0.44       0       (92)       1       LGEDNINVVEGHEQFISASK + 2->K [+0.04]         7       294       1094.81       2187.61       2188.03       -0.42       0       (94)       1       LGEDNINVVEGHEQFISASK + Acety1 (N-term); L->P [-16.04]         7       296       102.80       2203.59       2204.10       -0.50       0       (102)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (NR) [+57.05]         7       300       1110.30       2218.58       2219.07       -0.49       0       (105)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (NR) [+57.05]         7       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (NR) [+57.05]         8       alkaline phosphatase (EC 3.1.3.1)       precursor, intestinal - human       2       Check to include this hit in error tolerant search or archive report <td< td=""><td>✓ <u>195</u></td><td>805.17</td><td>1608.32</td><td>1608.65</td><td>-0.33</td><td>0</td><td>79</td><td>1</td><td>DSCQGDSGGPVVCSGK + Carbamidomethyl (C); Carbamidomethy</td></td<>	✓ <u>195</u>	805.17	1608.32	1608.65	-0.33	0	79	1	DSCQGDSGGPVVCSGK + Carbamidomethyl (C); Carbamidomethy
7       290       721.88       2162.09       0.52       0       (92)       1       LGEDNINVVEGHEQFISASK + Q->K [+0.04]         7       293       729.53       2185.58       2186.02       -0.44       0       (92)       1       LGEDNINVVEGHEQFISASK + Q->K [+0.04]         2       293       729.53       2185.58       2186.02       -0.44       0       (92)       1       LGEDNINVVEGHEQFISASK + L->H [+23.98]         2       294       1094.81       2187.61       2188.03       -0.42       0       (94)       1       LGEDNINVVEGHEQFISASK + Acety1 (N-term); L->P [-16.04]         2       296       1102.80       2203.59       2204.10       -0.50       0       (102)       1       LGEDNINVVEGHEQFISASK + G->V [+42.08]         2       300       1110.30       2218.58       2219.07       -0.49       0       (105)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (KR) [+57.05]         2       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (KR) [+57.05]         2       301       740.54       2218.58       256776       Total score: 342       Peptides matched: 9       alkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human <td>✓ 270</td> <td>988.30</td> <td>1974.58</td> <td>1973.95</td> <td>0.63</td> <td>0</td> <td>57</td> <td>1</td> <td>NINVVEGNEQFISASK + Biotinylated (N-term) [+226.29]</td>	✓ 270	988.30	1974.58	1973.95	0.63	0	57	1	NINVVEGNEQFISASK + Biotinylated (N-term) [+226.29]
Z       293       729.53       2185.58       2186.02       -0.44       0       (92)       1       LGEDNINVVEGNEQFISASK + L->H [+23.98]         Z       294       1094.81       2187.61       2188.03       -0.42       0       (94)       1       LGEDNINVVEGNEQFISASK + Acetyl (N-term); L->P [-16.04]         Z       296       1102.80       2203.59       2204.10       -0.50       0       (102)       1       LGEDNINVVEGNEQFISASK + Acetyl (N-term); L->P [-16.04]         Z       300       1110.30       2218.58       2219.07       -0.49       0       (105)       1       LGEDNINVVEGNEQFISASK + Carbanidomethyl (XR) [+57.05]         Z       300       110.30       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGNEQFISASK + Carbanidomethyl (XR) [+57.05]         Z       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGNEQFISASK + Carbanidomethyl (XR) [+57.05]         Z       alkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human       Z       Z       Check to include this hit in error tolerant search or archive report         Query       Observed       Mr(expt)       Mr(calc)       Delta       Miss Score       Rank       Peptide	✓ <u>289</u>	1081.77	2161.52	2162.05	-0.53	0	138	1	LGEDNINVVEGNEQFISASK
Z       294       1094.81       2187.61       2188.03       -0.42       0       (94)       1       LGEDNINVVEGHEQFISASK + Acetyl (N-term); L->P [-16.04]         Z       296       1102.80       2203.59       2204.10       -0.50       0       (102)       1       LGEDNINVVEGHEQFISASK + Acetyl (N-term); L->P [-16.04]         Z       300       1110.30       2218.58       2219.07       -0.49       0       (105)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (XR) [+57.05]         Z       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (XR) [+57.05]         Z       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGHEQFISASK + Carbanidomethyl (XR) [+57.05]         Z       Balkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human       2       Check to include this hit in error tolerant search or archive report         Query       Observed       Mr(calc)       Delta       Miss Score       Rak       Peptide         52       517.18       1032.34       1032.56       -0.22       0       67       2       GSSIFGLAPSK + S->G [-30.03]	✓ 290	721.88	2162.60	2162.09	0.52	0	(92)	1	LGEDNINVVEGNEQFISASK + Q->K [+0.04]
7       296       1102.80       2203.59       2204.10       -0.50       0       (102)       1       LGEDNINVVEGHEQFISASK + G->V [+42.08]         2       300       1110.30       2218.58       2219.07       -0.49       0       (105)       1       LGEDNINVVEGHEQFISASK + Carbanidomethy2       (KR) [+57.05]         2       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGHEQFISASK + Carbanidomethy2       (KR) [+57.05]         2       9       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGHEQFISASK + Carbanidomethy2       (KR) [+57.05]         2       9       alkaline phosphatase       [EC 3.1.3.1]       precursor, intestinal - human       9         2       Check to include this hit in error tolerant search or archive report       0       Query       Observed       Mr(calc)       Delta       Miss Score       Rak       Peptide         52       517.18       1032.34       1032.56       -0.22       0       67       2       GSSIFGLAPSK + S->G [-30.03]	✓ 293	729.53	2185.58	2186.02	-0.44	0	(92)	1	LGEDNINVVEGNEQFISASK + L->H [+23.98]
7       300       1110.30       2218.58       2219.07       -0.49       0       (105)       1       LGEDNINVVEGHEQFISASK + Carbamidomethyl (KR) [+57.05]         2       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNINVVEGHEQFISASK + Carbamidomethyl (KR) [+57.05]         .       PAHUT       Mass: 56776       Total score: 342       Peptides matched: 9         alkaline phosphatase (EC 3.1.3.1)       precursor, intestinal - human         Check to include this hit in error tolerant search or archive report         Query       Observed       Mr(calc)       Delta       Miss Score       Rak       Peptide         52       517.18       1032.34       1032.56       -0.22       0       67       2       GSSIFGLAPSK + S->G [-30.03]	✓ <u>294</u>	1094.81	2187.61	2188.03	-0.42	0	(94)	1	LGEDNINVVEGNEQFISASK + Acetyl (N-term); L->P [-16.04]
Z       301       740.54       2218.58       2219.07       -0.49       0       (93)       1       LGEDNTRVVEGNEQFISASK + Carbamidomethyl (KR) [+57.05]         .       PAHUI       Mass: 56776       Total score: 342       Peptides matched: 9         alkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human         Z       Check to include this hit in error tolerant search or archive report         Query       Observed       Mr(calc)       Delta       Miss Score       Rank       Peptide         52       517.18       1032.34       1032.56       -0.22       0       67       2       GSSIFGLAPSK + S->G [-30.03]	✓ <u>296</u>	1102.80	2203.59	2204.10	-0.50	0	(102)	1	LGEDNINVVEGNEQFISASK + G->V [+42.08]
<ul> <li><u>PAHUI</u> Mass: 56776 Total score: 342 Peptides matched: 9 alkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human</li> <li>Check to include this hit in error tolerant search or archive report</li> <li>Query Observed Mr(expt) Mr(calc) Delta Miss Score Rank Peptide</li> <li>52 517.18 1032.34 1032.56 -0.22 0 67 2 GSSIFGLAPSK + S-&gt;G [-30.03]</li> </ul>	✓ <u>300</u>	1110.30	2218.58	2219.07	-0.49	0	(105)	1	LGEDNINVVEGNEQFISASK + Carbamidomethyl (KR) [+57.05]
alkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human Check to include this hit in error tolerant search or archive report Query Observed Mr(expt) Mr(calc) Delta Miss Score Rank Peptide <u>52</u> 517.18 1032.34 1032.56 -0.22 0 67 2 GSSIFGLAPSK + S->G [-30.03]	✓ <u>301</u>	740.54	2218.58	2219.07	-0.49	0	(93)	1	LGEDNINVVEGNEQFISASK + Carbamidomethyl (KR) [+57.05]
alkaline phosphatase (EC 3.1.3.1) precursor, intestinal - human Check to include this hit in error tolerant search or archive report Query Observed Mr(expt) Mr(calc) Delta Miss Score Rank Peptide <u>52</u> 517.18 1032.34 1032.56 -0.22 0 67 2 GSSIFGLAPSK + S->G [-30.03]	PAHIIT		Mass: 56776	Total so	ore: 342	Pen	tides m	atched	: 9
- Query Observed Mr(expt) Mr(calc) Delta Miss Score Rank Peptide <u>52</u> 517.18 1032.34 1032.56 -0.22 0 67 2 GSSIFGLAPSK + S->G [-30.03]									
52 517.18 1032.34 1032.56 -0.22 0 67 2 GSSIFGLAPSK + S->G [-30.03]	Check t	co include t	his hit in	error tole:	ant sea	rch or	archi	ve repo	rt
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	<u> </u>	F22 40	1000.05	1000 57	0.00	<u> </u>	1500	1	CCCTECI ADCH

There are several matches to the same peptide

LGEDNINVVEGNEQFISASK with a variety of modifications. The first, Q->K, is identical to no modification. Although the match to Query 296 is shown as G->V, we would prefer to assign the mass change of 42 Da to N-term acetylation.



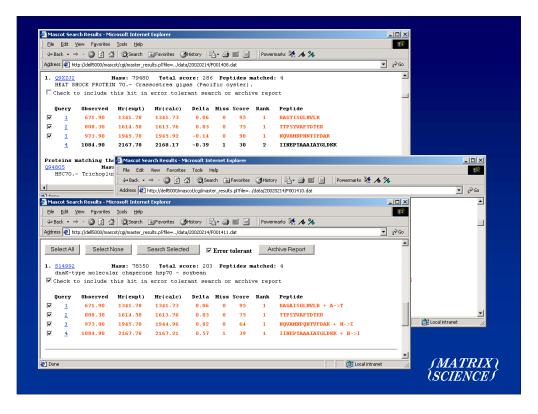
Looking at the details for this match

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¥	b	b++	b*	b* <sup>++</sup>	Ъ <sup>0</sup>	b <sup>0++</sup>	Seq.	У	y++	у*	y* <sup>++</sup>	y <sup>0</sup>	y <sup>0++</sup>	#	
1	156.10	78.56					L							20	
2	213.12	107.07					G	2049.97	1025.49	2032.95	1016.98	2031.96	1016.49	19	
3	342.17	171.59			324.16	162.58	Е	1992.95	996.98	1975.92	988.47	1974.94	987.97	18	
4	457.19	229.10			439.18	220.10	D	1863.91	932.46	1846.88	923.95	1845.90	923.45	17	
5	571.24	286.12	554.21	277.61	553.23	277.12	Ν	1748.88	874.94	1731.86	866.43	1730.87	865.94	16	
6	684.32	342.66	667.29	334.15	666.31	333.66	Ι	1634.84	817.92	1617.81	809.41	1616.83	808.92	15	
7	798.36	399.69	781.34	391.17	780.35	390.68	Ν	1521.75	761.38	1504.73	752.87	1503.74	752.38	14	
8	897.43	449.22	880.41	440.71	879.42	440.21	V	1407.71	704.36	1390.69	695.85	1389.70	695.35	13	
9	996.50	498.75	979.47	490.24	978.49	489.75	V	1308.64	654.83	1291.62	646.31	1290.63	645.82	12	
.0	1125.54	563.28	1108.52	554.76	1107.53	554.27	Е	1209.58	605.29	1192.55	596.78	1191.56	596.29	11	
1	1182.56	591.79	1165.54	583.27	1164.55	582.78	G	1080.53	540.77	1063.51	532.26	1062.52	531.76	10	
2	1296.61	648.81	1279.58	640.29	1278.60	639.80	Ν	1023.51	512.26	1006.48	503.75	1005.50	503.25	9	
3	1425.65	713.33	1408.62		1407.64		Е	909.47	455.24	892.44	446.72	891.46	446.23	8	
-	1553.71		1536.68		1535.70	768.35	Q	780.43	390.72	763.40	382.20	762.42	381.71	7	
			1683.75		1682.77	841.89	F	652.37	326.69	635.34	318.17		317.68		
.6	1813.86	907.43	1796.83	898.92	1795.85	898.43	Ι	505.30	253.15	488.27	244.64	487.29	244.15	5	
_			1883.87		1882.88	941.95	S	392.21	196.61				187.61	4	
8	1971.93	986.47	1954.90	977.96	1953.92	977.46	Α	305.18	153.10	288.16	144.58	287.17	144.09	3	
9	2058.96	1029.98	2041.94	1021.47	2040.95	1020.98	S	234.15	117.58	217.12	109.06	216.13	108.57	2	
0							к	147.11	74.06	130.09	65.55			1	

We see that the fragment ions have a quantitative neutral loss of water. Is this behaviour well known for acetylated Leu?

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_		to include t				-			
Q	uery	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
7	<u>6</u>	403.13	804.25	804.41	-0.16	0	65	1	SAASLNSR
•	<u>15</u>	431.63	861.24	861.43	-0.19	0	(51)	1	SAASLNSR + Carbamidomethyl (N-term) [+57.05]
7	<u>74</u>	559.15	1116.29	1116.52	-0.23	0	56	1	SIVHPSYNSN
•	<u>90</u>	577.17	1152.32	1152.57	-0.25	0	87	1	SSGTSYPDVLK
•	<u>93</u>	584.67	1167.33	1167.57	-0.25	0	97	1	VCNYVSWIK + Carbamidomethyl (C)
•	100	598.18	1194.34	1194.58	-0.24	0	(68)	1	SSGTSYPDVLK + Acetyl (N-term)
•	107	606.19	1210.35	1210.55	-0.20	0	(57)	1	SSGTSYPDVLK + Acetyl (N-term); S->C [+16.06]
•	173	745.72	1489.43	1489.73	-0.31	0	84	1	LQGIVSWGSGCAQK + Carbamidomethyl (KR) [+57.05]
1	195	805.17	1608.32	1608.65	-0.33	0	79	1	DSCQGDSGGPVVCSGK + Carbamidomethyl (C); Carbamidometh
•	270	988.30	1974.58	1973.95	0.63	0	57	1	NINVVEGNEQFISASK + Biotinylated (N-term) [+226.29]
•	289	1081.77	2161.52	2162.05	-0.53	0	138	1	LGEDNINVVEGNEQFISASK
1	290	721.88	2162.60	2162.09	0.52	0	(92)	1	LGEDNINVVEGNEQFISASK + Q->K [+0.04]
•	293	729.53	2185.58	2186.02	-0.44	0	(92)	1	LGEDNINVVEGNEQFISASK + L->H [+23.98]
•	294	1094.81	2187.61	2188.03	-0.42	0	(94)	1	LGEDNINVVEGNEQFISASK + Acetyl (N-term); L->P [-16.04]
1	296	1102.80	2203.59	2204.10	-0.50	0	(102)	1	LGEDNINVVEGNEQFISASK + G->V [+42.08]
•	300	1110.30	2218.58	2219.07	-0.49	0	(105)	1	LGEDNINVVEGNEQFISASK + Carbamidomethyl (KR) [+57.05]
•	<u>301</u>	740.54	2218.58	2219.07	-0.49	0	(93)	1	LGEDNINVVEGNEQFISASK + Carbanidomethyl (KR) [+57.05]
F	AHUI	1	Mass: 56776	Total sc	ore: 342	Pep	tides n	atched	: 9
		ne phosphat							-
C:	heck	to include t	his hit in	error tole:	ant sea	rch or	archi	ve repo	rt
Q	uery	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Rank	Peptide
	<u>52</u>	517.18	1032.34	1032.56	-0.22	0	67	2	GSSIFGLAPSK + S->G [-30.03]
	- 00	F22 40	1000.05	1000 57	0.00	- <b>•</b>	1503		CONTROL NOW

This suggests that a possible assignment of +24 Da for query 293 is prompt loss of water: 42-18=24???



Finally, a slightly artificial illustration of finding a point mutation in the primary sequence. We can lose the match to query 1 by changing the taxonomy to 'green plants', which excludes both entries containing the correct peptide. If we now do an error tolerant search, the correct match is recovered via the substitution A->T

### **Error Tolerant Search of Uninterpreted MS/MS Data**

- Successfully locate mass differences
- Difficult to report correct assignment
- Limited to proteins which have at least one good peptide match ... not very useful for (say) MHC peptides
- Next step: hybrid of Sequence Tag & MS/MS Ions Search?

To summarise, the error tolerant search of uninterpreted MS/MS data is a powerful way of finding additional peptide matches. However, it is very difficult to provide a chemically credible assignment for many of the observed mass differences. Some expertise in mass spectrometry and protein chemistry is required to review and correct the reported assignments.

*(MATRIX)* (*SCIENCE*)

