

Modifications are a very important topic in database searching.

In some cases, the main focus of a study is to characterise post translational modifications, which may have biological significance. Phosphorylation would be a good example.

In other cases, the modification may not be of interest in itself, but you need to allow for it in order to get a match. Oxidation during sample preparation would be an example.

And, of course, many methods of quantitation involve modifications containing isotopic labels

Some sequence variants, such as the substitution of one residue by another, are equivalent to modifications, and can be handled in a similar way

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Comprehensive and accurate information about post translational and chemical modifications is an essential factor in the success of protein identification. In Mascot, we take our list of modifications from Unimod, which is an on-line modifications database.

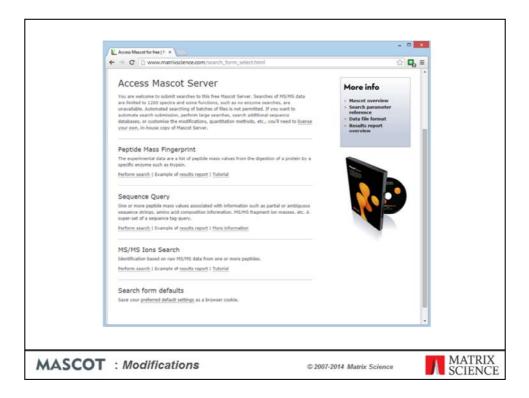
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Accession #	56		PSI-MS Name	Acetyl:2H(3)	Interim Name	AcetyLheavy
Description	Acetate 1	abeling reagent (N	i-term 6 K) (heavy form, +3ar	mu)		
Alt. Description	N-trideut	eriumacetoxy.				
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Curator	penner	Last Modified	2006-10-16 10:02:50		Verified	Yes
Back to list						

There are other lists of modifications on the web, like DeltaMass on the ABRF web site and RESID from the EBI, but none is as comprehensive as Unimod

Mass values are calculated from empirical chemical formulae, eliminating the most common source of error. Specificities can be defined in ways that are useful in database searching, and there is the option to enter mass-spec specific data, such as neutral loss information. This screen shot shows one of the better annotated entries, I can't pretend that all of them are this detailed. Nevertheless, it is a very useful, public domain resource that beats having to create your own list in an Excel spreadsheet or on the back of an envelope.



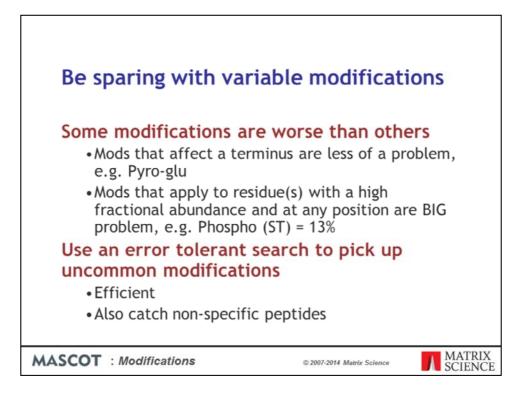
If you go to the help page, there is a link to download the contents of Unimod as a Mascot modifications file. This is the easiest way to keep the modifications list on an in-house Mascot server up-to-date



Here is a tip. The default list of modifications displayed in the Mascot search form is a short list, containing only the most common mods. If you want to see the complete list of mods, and you are using Mascot 2.2 or earlier, you need to follow the link at the bottom of the search form selection page

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100000000000000000000000000000000000000	search form defaults		
Database	SwissProt - NCBlor contaminants cRAP		
Taxonomy	All entries	•	
Enzyme	Trypsin •		
Allow up to	1 • missed cleavages		
Fixed modifications	2-dimethylsuccinyl (C) 2-monomethylsuccinyl (C) 2-minobenzyl (Y) 2-succinyl (C) 2HPG (R)	Î	
Variable modifications		ii.	
Show all mods.	8		
Quantitation	None •		
Peptide tol. ±	12 Da • #13C 0 •		
MS/MS tol. ±	0.6 Da •		
Peptide charge	[1+ · ·		
Monoisotopic	* Average 0		
Data format	Mascot generic • (MS/MS only)		
Instrument	Default • (HS/HS only)		
Decoy	0		
Error Inlarant	10		

Check the box for Show all mods, then choose Save. This still sets the default state of the checkbox in Mascot 2.3, but we decided to place the checkbox on the search form, so as to make it easier to swap between the short and long lists.

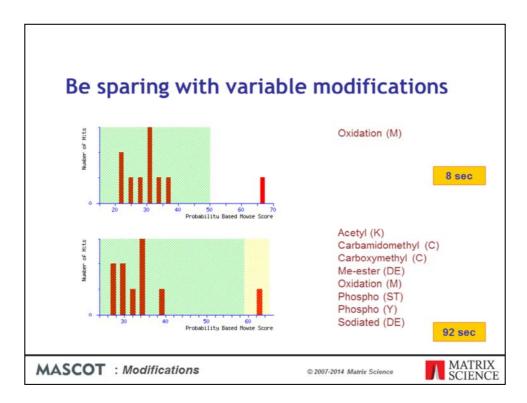


It is extremely important that you do not choose more than the absolute minimum number of variable modification in a search. We talked about this in an earlier presentation, but it is worth repeating.

Variable or differential or non-quantitative modifications are expensive, in the sense that they increase the time taken for a search and reduce its specificity. This is because the software has to permute out all the possible arrangements of modified and unmodified residues that fit to the peptide molecular mass. As more and more modifications are considered, the number of combinations and permutations increases geometrically. The so-called combinatorial explosion.

Some variable modifications are worse than others. Modifications that only apply to a terminus, especially if they only apply when particular residue is at the terminus, like pyro-glu, make little difference to the number of peptides to be tested. The problem modifications are the ones that apply to residues in any position, especially if they apply to multiple residues, like phosphorylation.

Unless you have enriched the sample in a particular PT-mod, e.g IMAC for phosphopeptides, it is usually not a good idea to try and catch PT-mods in a first pass search. Better to use a second pass search, which we call an error tolerant search, to catch the low abundance mods. We will come back to this later.



To illustrate this point. This search of a single MS/MS spectrum, using one variable mod, gives a nice, statistically significant match.

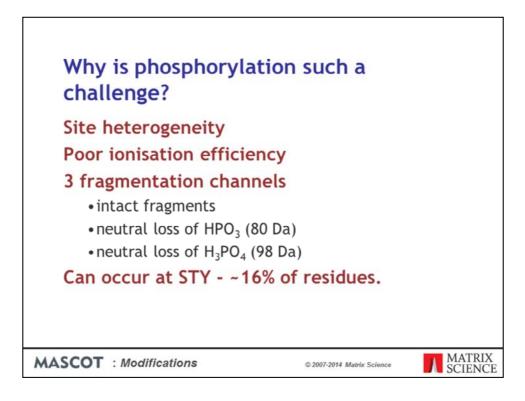
If the search is repeated with 8 mods, the match is the same, with an identical score, but now it is barely significant.

All of these mods have effectively increased the size of the database by a factor of 30

What's worse, the search takes over 10 times as long!

So, use variable mods sparingly. You'll get better results and faster.

By the way, the yellow region in the histogram indicates scores above the homology but below the identity thresholds. You will only see these regions highlighted in an MS/MS search report if it is a search of a single spectrum.

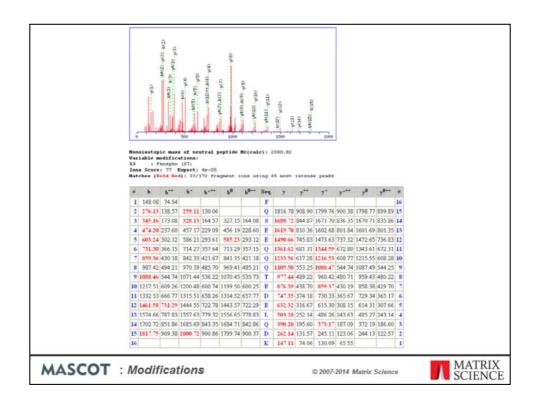


Of all post-translational modifications, phosphorylation is one of the most interesting and also one of the most difficult. Why is it such a challenge?

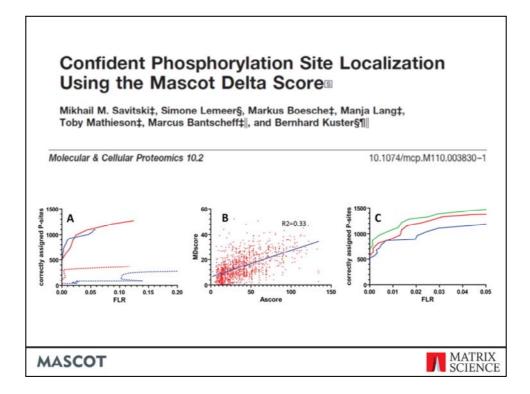
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Search Pat         13.6         47         -0.1469         K. UFLEELTPROQUER.S           12.6         58         -0.1366         K. SSS01PT0PPURDONEK.S
Type of sea         12.3         64         -0.2007         K.SSOUF/UPY_LSPIAN.0           Enzyme         11.9         71         -0.1635         K.YLLCVGETLMERDEEK.R

Lets look at an example or two.

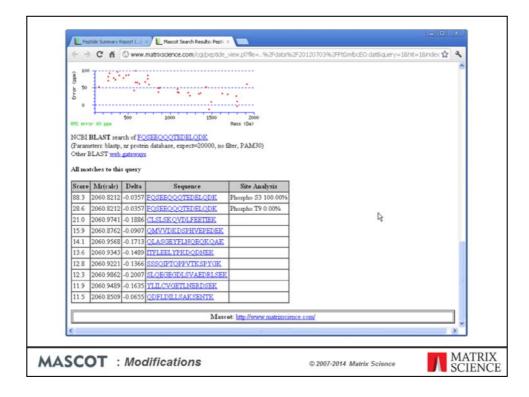
One of the most common phosphopeptides comes from the milk protein, beta casein. There are two potential phosphorylation sites, S and T, but only one is modified. Because the two sites are widely separated, the two arrangements get very different scores.



Beautiful spectrum; long run of y ions; move site to T9 and many matches would disappear



Mascot 2.4 reports site localisation probabilities using the delta score method published in MCP by Bernard Kuster's group. They analysed a collection of synthetic analogs of real phosphopeptides and determined what score difference was required to determine the correct site with an error rate of (say) 5%. Because we don't expect everyone to calibrate their data in this way, we have made the calculation slightly more conservative. A score difference of 10 would give approximately 90% probability that the higher scoring arrangement was correct.

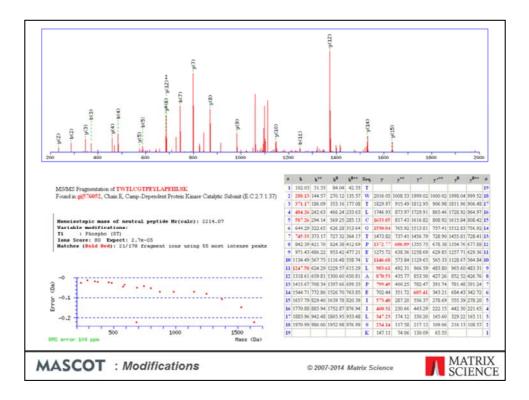


A very large score difference such as the one we were just looking at gives 100% likelihood that the phosphate is on S3.

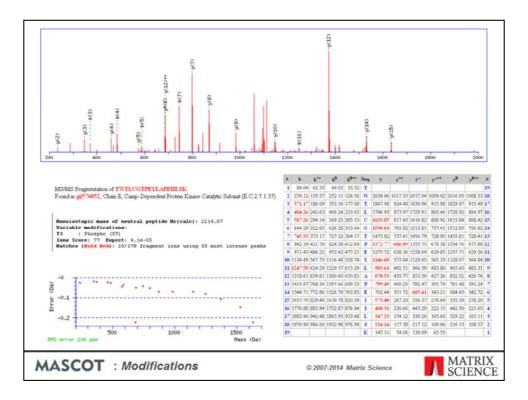
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However, casein peptides are unusually easy to analyse. Here is a more typical example of what you can expect to find - a strong match to a phosphopeptide from a protein kinase.

There is little to choose in terms of score between having the phosphate on T1 or T3.



We can see why there is little difference in score between placing the phosphate on T1 or T3. There is just one extra matched peak, and in probability terms, there isn't a huge difference between 20 matches using 55 experimental peaks and 21. However, if you had to choose one or the other, you'd probably go for T1



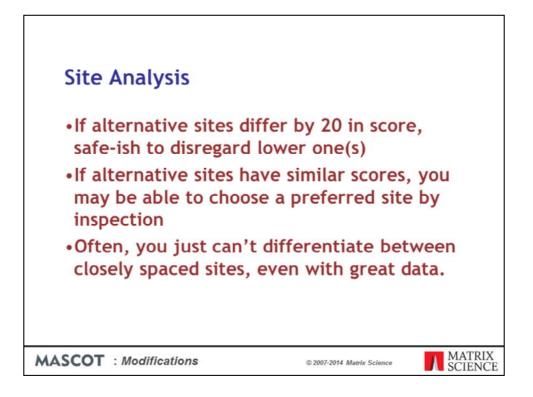
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76.9 38.7 18.0 12.6 12.6	2214.0683 2214.0683 2214.0683 2214.0044 2214.0044 2214.0044 2214.0044	-0.2750 -0.2750 -0.2750 -0.2111 -0.2111 -0.2111	TWTLCGTPEYLAPEIILSK TWTLCGTPEYLAPEIILSK TWTLCGTPEYLAPEIILSK GGSGMLTLGIPSSPGVPAELSK GGSGMLTLGIPSSPGVPAELSK	Phospho T3 30.83% Phospho T7 0.00%		
76.9 38.7 18.0 12.6 12.6 12.6	2214.0683 2214.0683 2214.0683 2214.0044 2214.0044 2214.0044 2214.0044	-0.2750 -0.2750 -0.2750 -0.2111 -0.2111 -0.2111 -0.2111	TWILCOTPEYLAPEILSK TWILCOTPEYLAPEILSK TWILCOTPEYLAPEILSK GOSGMUTLGIPSSPGVPAELSK GOSGMUTLGIPSSPGVPAELSK GOSGMUTLGLPSSPGVPAELSK	Phospho T3 30.83% Phospho T7 0.00%		
76.9 38.7 18.0 12.6 12.6 12.6	2214.0683 2214.0683 2214.0683 2214.0044 2214.0044 2214.0044 2214.0044 2214.0044	-0.2750 -0.2750 -0.2750 -0.2111 -0.2111 -0.2111 -0.2111 -0.2111	TWILCOTPEYLAPEILSK TWILCOTPEYLAPEILSK TWILCOTPEYLAPEILSK GGSGMLILGIPSSPGVPAELSK GGSGMLILGIPSSPGVPAELSK GGSGMLILGIPSSPGVPAELSK	Phospho T3 30.83% Phospho T7 0.00%		
76.9 38.7 18.0 12.6 12.6 12.6 12.6 11.9	2214.0683 2214.0683 2214.0683 2214.0044 2214.0044 2214.0044 2214.0044 2214.0044	-0.2750 -0.2750 -0.2750 -0.2111 -0.2111 -0.2111 -0.2111 -0.2111	TWILCOTPEYLAPEIILSK TWILCOTPEYLAPEIILSK TWILCOTPEYLAPEIILSK GOSGMITILGIPSSPGVPAELSK GOSGMITILGIPSSPGVPAELSK GOSGMITILGIPSSPGVPAELSK GOSGMITILGIPSSPGVPAELSK	Phospho T3 30.83% Phospho T7 0.00%		
76.9 38.7 18.0 12.6 12.6 12.6 12.6 11.9	2214.0683 2214.0683 2214.0683 2214.0044 2214.0044 2214.0044 2214.0044 2214.0044	-0.2750 -0.2750 -0.2750 -0.2111 -0.2111 -0.2111 -0.2111 -0.2111	TWILCOTPEYLAPEIILSK TWILCOTPEYLAPEIILSK GOSOMITLOPSSPOUPAELSK GOSOMITLOPSSPOUPAELSK GOSOMITLOPSSPOUPAELSK GOSOMITLOPSSPOUPAELSK GOSOMITLOPSSPOUPAELSK GOSOMITLOPSSPOUPAELSK	Phospho T3 30.83% Phospho T7 0.00%	com/	

The delta score site analysis suggests 70% probability on T1 and 30% on T3 ... much less clear cut. We can't be confident which site is modified, or whether there is a mixture of both isoforms. But, we can be confident it is not on T7 or Y10 because the score drops dramatically, and these are assigned 0% probability.

Sometimes, it is worth looking at the sequence annotations to see whether these are known phosphorylation sites. If the database sequence doesn't have detailed annotations, you can follow the BLAST link to try and match the peptide to an entry from a better annotated database. In this case, we're searching SwissProt, so we can go straight to the protein view report

TT       BKO2       PLOS PLOS ING       Phosphothreonine (By similarity). = T1         TT       BKO2       PLOS PLOS ING       Phosphothreonine (By similarity). = T3         TT       BKO2       PLOSPhothreonine (By similarity). = T3         TT       BKO2       PLOSPhothreonine (By similarity). = T3         TT       BKO2       PLOSPhothreonine (By similarity). = T3         TT       BKO2       PLOSPhothreonine.         TT       BKO2       PLOSPhothreonine.         TT       BKO2       PLOSPhothreonine.         TT       BKO2       PLOSPhothreonine.         TT       BKD2       PLOSPhothreonine.         TT       BKD2       PLOSPhothreonine.         TT       STAND       ST         TC       CONFLICT       202       202         T       CONFLICT       204       T -> N (In Ref. 4; Ak sequence).         TC       CONFLICT       205       206       L -> S (In Ref. 4; Ak sequence).         TT       CONFLICT       207       N -> D (In Ref. 4; Ak sequence).         TT       STEAND       44       52         TT       STEAND       54       63         TT       STEAND       54       63 <td< th=""><th>22.5</th><th>→ C fi</th><th></th><th></th><th>ce.com/cgi/protein_view.pl?file=%2Fdata%2F20120704%2FFtGmlfewT.dat8ht=KAPCA_BOVIN8db, 🏠   🔧</th></td<>	22.5	→ C fi			ce.com/cgi/protein_view.pl?file=%2Fdata%2F20120704%2FFtGmlfewT.dat8ht=KAPCA_BOVIN8db, 🏠   🔧
<pre>TT MCO_RES 196 196 Phosphothreonine (By pimilarity). = T1 MCO_RES 196 196 Phosphothreonine by PPKN, = T3 TT MCO_RES 202 202 Phosphothreonine (By similarity). = T3 TT MCO_RES 309 309 Phosphoterine. TT LUTD 3 3 Phosphoterine. TT LUTD 3 3 Phosphoterine. TT LUTD 4 3 3 Phosphoterine. TT CONFLICT 202 204 204 F -&gt; 0 (in Ref. 4) AA sequence). TT CONFLICT 204 204 L -&gt; 0 (in Ref. 4) AA sequence). TT CONFLICT 205 207 N -&gt; D (in Ref. 4) AA sequence). TT CONFLICT 205 207 N -&gt; D (in Ref. 4) AA sequence). TT CONFLICT 207 207 N -&gt; D (in Ref. 4) AA sequence). TT CONFLICT 206 L -&gt; S (in Ref. 4) AA sequence). TT TT TURN 64 65 TT STRAND 54 63 TT STRAND 54 63 TT STRAND 54 66 TT STRAND 54 66 TT STRAND 107 112 TT STRAND 1</pre>	11	HOD PLJ	120	17	ruosphoraleonine (by similaricy).
T     MCO_RES     190     190     Phosphothconing: by PPFK1.       T     MCO_RES     202     CO     Phosphothconing: (by similarity). = T3       T     MCO_RES     303     N-expiratoryl glycine.       T     MUTAGN     3     N-expiratoryl glycine.       T     MCO_RES     202     T -> N (in Ref. 4) AA sequence).       T     COMFLICT     206     206     L -> S (in Ref. 4) AA sequence).       T     COMFLICT     206     206     L -> S (in Ref. 4) AA sequence).       T     COMFLICT     206     206     L -> S (in Ref. 4) AA sequence).       T     COMFLICT     206     206     L -> S (in Ref. 4) AA sequence).       T     COMFLICT     206     206     L -> S (in Ref. 4) AA sequence).       T     T MULX     16     32       T     TSTRAMD     46     66       TT     STRAMD     107     112       TT     STRAMD					
TT       NOC_RES       202       202       Phosphostine.         TI       NOC_RES       319       Phosphostine.         FT       LIPID       2       2       N-syristoyl glycine.         FT       NUTLOCT       202       202       T -> N (in Ref. 4; Ak sequence).         FT       CONFLICT       202       203       T -> N (in Ref. 4; Ak sequence).         FT       CONFLICT       206       204       E -> N (in Ref. 4; Ak sequence).         FT       CONFLICT       206       204       E -> N (in Ref. 4; Ak sequence).         FT       CONFLICT       206       204       E -> N (in Ref. 4; Ak sequence).         FT       CONFLICT       206       204       L -> N (in Ref. 4; Ak sequence).         FT       CONFLICT       206       204       L -> S (in Ref. 4; Ak sequence).         FT       CONFLICT       206       204       L -> S (in Ref. 4; Ak sequence).         FT       CONFLICT       206       204       K => S (in Ref. 4; Ak sequence).         FT       STEND       14       30       31         FT       NELIX       14       30       31         FT       STEND       14       12       31					
FT       MOD_RES       339       339       Phosphoserine.         FT       LIPTO       2       N-spristoyl glycine.         FT       MTTAGEN       3       N-spristoyl glycine.         FT       CONFLICT       202       T -s N (in Ref. 4; AA sequence).         FT       CONFLICT       205       204       T -s N (in Ref. 4; AA sequence).         FT       CONFLICT       205       204       L -s S (in Ref. 4; AA sequence).         FT       CONFLICT       205       204       L -s S (in Ref. 4; AA sequence).         FT       CONFLICT       205       204       L -s S (in Ref. 2; AA sequence).         FT       TSTRAMD       44       52         FT       STRAMD       44       52         FT       STRAMD       56       64         FT       STRAMD       60       64         FT       STRAMD       107       112         FT       STRAMD       170       112         FT       STRAMD       173       175         FT       STRAMD       173       175         FT       STRAMD       173       175         FT       STRAMD       163       175					Phosphothreonine; by PDPK1. = T3
PT       LiPID       2       2       N-mprintopi glycime.         PT       NUTAGN       3       N-b) No myristoplation.         PT       CONFLICT       202       202       T -> N (in Ref. 4; Ak sequence).         PT       CONFLICT       206       204       E -> S (in Ref. 4; Ak sequence).         PT       CONFLICT       206       206       L -> S (in Ref. 4; Ak sequence).         PT       CONFLICT       206       206       L -> S (in Ref. 4; Ak sequence).         PT       CONFLICT       206       206       L -> S (in Ref. 4; Ak sequence).         PT       CONFLICT       207       N -> D (in Ref. 4; Ak sequence).         PT       CONFLICT       206       206         PT       CONFLICT       207       N -> D (in Ref. 4; Ak sequence).         PT       CONFLICT       206       206       L -> S (in Ref. 4; Ak sequence).         PT       CONFLICT       206       206       L -> S (in Ref. 4; Ak sequence).         PT       STRIND       44       52       String (in Ref. 4; Ak sequence).         PT       STRIND       10       112       String (in Ref. 4; Ak sequence).         PT       STRIND       11       12       String (in Ref. 4; Ak seq					
FT       NUTJAČEN       3       3       N-5D: No myristopletion.         FT       CONFLICT       204       204       F. > Q (in Ref. 4) AA sequence).         FT       CONFLICT       204       204       E -> Q (in Ref. 4) AA sequence).         FT       CONFLICT       206       L -> Q (in Ref. 4) AA sequence).         FT       CONFLICT       206       L -> Q (in Ref. 4) AA sequence).         FT       CONFLICT       207       N -> D (in Ref. 4) AA sequence).         FT       FC       CONFLICT       206       L -> Q (in Ref. 4) AA sequence).         FT       CONFLICT       207       N -> D (in Ref. 4) AA sequence).         FT       STEAMD       44       52         FT       STEAMD       54       63         FT       STEAMD       67       64         FT       STEAMD       67       76         FT       STEAMD       67       76         FT       STEAMD       107       112         FT       STEAMD       107       112         FT       STEAMD       107       112         FT       STEAMD       107       122         FT       STEAMD       107       122 </td <td></td> <td></td> <td></td> <td></td> <td></td>					
T         CONFLICT         202         202         T -> N (in Ref. 4) AM sequence).           T         CONFLICT         204         E -> S (in Ref. 4) AM sequence).           FT         CONFLICT         206         204         E -> S (in Ref. 4) AM sequence).           FT         CONFLICT         206         204         E -> S (in Ref. 4) AM sequence).           FT         CONFLICT         206         206         L -> S (in Ref. 4) AM sequence).           FT         CONFLICT         207         N -> D (in Ref. 4) AM sequence and 3; AM sequence).           FT         TT STEAMD         44         52           FT         TSTEAMD         54         63           FT         STEAMD         64         66           FT         STEAMD         107         112           FT         STEAMD         107					
TT CONFLICT 204 204 E -> Q (in Ref. 4; AA sequence). TT CONFLICT 205 207 207 Los A (in Ref. 4; AA sequence). TT CONFLICT 205 207 N -> D (in Ref. 4; AA sequence). TT CONFLICT 205 207 N -> D (in Ref. 4; AA sequence). TT ELLIX 16 22 TT ELLIX 16 22 TT STRAND 54 52 TT STRAND 54 63 TT STRAND 54 66 TT STRAND 69 76 TT STRAND 69 76 TT STRAND 107 112 TT STRAND 107 112 STRONGE 107 107 STRONGE 107 107 STR					
T         CONFLICT         206         206         L -> S (in Ref. 4; A A sequence).           FT         CONFLICT         207         N -> D (in Ref. 2; AA sequence and 3; AA sequence).           FT         CONFLICT         207         N -> D (in Ref. 2; AA sequence and 3; AA sequence).           FT         RELIX         1         43           FT         STEAMD         54           FT         STEAMD         54           FT         STEAMD         54           FT         STEAMD         59           FT         TTEND         64           FT         STEAMD         57           FT         STEAMD         107           FT         STEAMD         107           FT         STEAMD         107           FT         STEAMD         173           STEAMD         173         175           FT         STEAMD         173           FT         STEAMD         173           FT         STEAMD					
T         CONTLICT         207         207         N → D         (in Ref. 2; AA sequence and 3; AA           FT         HELIX         16         32           FT         HELIX         16         32           FT         HELIX         41         43           FT         STRAND         44         52           FT         STRAND         54         63           FT         STRAND         54         63           FT         STRAND         54         63           FT         HELIX         64         64           FT         STRAND         54         63           FT         HELIX         65         96           FT         HELIX         77         62           FT         STRAND         114         122           FT         STRAND         114         122           FT         STRAND         173         175           FT         STRAND         173         175           FT         STRAND         173         175           FT         HELIX         100         103           FT         HELIX         264         273					
T         sequence).           FH         HLLIX         16           FT         HKLIX         41           FT         HKLIX         41           FT         STEAMD         44           FT         STEAMD         44           FT         STEAMD         64           FT         STEAMD         69           FT         STEAMD         69           FT         STEAMD         107           FT         STEAMD         103           FT         STEAMD         103           FT         STEAMD         103           FT         HELIX         200           FT         STEAMD         103           FT         HELIX         200           FT         HELIX         200           STEAMD         303 </td <td></td> <td></td> <td></td> <td></td> <td></td>					
PT       HELIX       16       32         PT       HELIX       41       43         PT       STRAND       44       52         PT       STRAND       54       63         PT       STRAND       64       64         PT       STRAND       64       66         PT       STRAND       69       64         PT       STRAND       69       64         PT       STRAND       69       64         PT       STRAND       107       102         PT       STRAND       114       122         PT       HELIX       140       160         PT       STRAND       113       160         PT       STRAND       131       103         PT       STRAND       173       175         PT       STRAND       131       103         PT       HELIX       203       205         PT       HELIX       264       273         PT       HELIX       264       273         PT       HELIX       264       280         SCOUCHAUS       SIALA       SIALA       SIALA		CONFLICT	287	287	N -> D (in Ref. 2; A& sequence and 3; A&
FT       HELIZ       41       43         FT       STRAND       44       52         FT       STRAND       54       63         FT       STRAND       54       63         FT       STRAND       54       63         FT       STRAND       54       66         FT       STRAND       67       76         FT       STRAND       67       76         FT       STRAND       107       112         FT       STRAND       107       12         FT       STRAND       103       114         FT       HELIX       203       205         FT       HELIX       200       201         FT       HELIX       204       253         FT       HELIX       204       253         FT       HELIX       2050       193					sequence).
TT       STRAND       44       S2         TSTRAND       54       63         FT       STRAND       54       63         FT       STRAND       64       64         FT       STRAND       69       76         FT       STRAND       69       76         FT       STRAND       69       76         FT       HELIX       70       82         FT       HELIX       10       12         FT       STRAND       14       120         FT       HELIX       140       121         FT       HELIX       141       160         FT       STRAND       173       175         FT       STRAND       173       175         FT       HELIX       200       205         FT       HELIX       203       205         FT       HELIX       204       214         FT       HELIX       204       214         FT       HELIX       205       203         FT       HELIX       206       209         FT       HELIX       206       208         SEQUENCE       3	FΤ				
TT       TSTRAND       54       63         TT       TURN       64       66         FT       STRAND       69       76         FT       STRAND       69       76         FT       STRAND       69       76         FT       STRAND       69       76         FT       STRAND       107       112         FT       STRAND       131       122         FT       STRAND       173       173         FT       STRAND       173       173         FT       HELIX       208       201         FT       HELIX       244       253         FT       HELIX       264       279         FT       HELIX       303       307					
PT       TURN       64       66         PT       STEAMD       69       76         PT       HELIX       77       82         PT       HELIX       76       84         PT       STEAMD       107       112         PT       HELIX       141       160         PT       STEAMD       173       175         PT       STEAMD       173       175         PT       STEAMD       131       163         PT       HELIX       200       205         PT       HELIX       200       205         PT       HELIX       200       201         PT       HELIX       200       203         PT       HELIX       206       290         PT       HELIX       303       307         PT       HELIX       364       340 <t< td=""><td>FT</td><td></td><td></td><td></td><td></td></t<>	FT				
FT         STRAND         60         76           FT         HELIX         77         82           FT         HELIX         66         96           FT         HELIX         86         96           FT         STRAND         107         112           FT         STRAND         114         122           FT         HELIX         141         120           FT         HELIX         141         160           FT         HELIX         170         173           FT         HELIX         100         173           FT         HELIX         100         174           FT         HELIX         100         174           FT         HELIX         203         105           FT         HELIX         204         203           FT         HELIX         204         203           FT         HELIX         206         209           FT         HELIX         206         200           FT         HELIX         206         200           FT         HELIX         206         200           FT         HELIX         303					
FT       HELIX       77       62         FT       HELIX       66       96         FT       STRAND       107       112         FT       STRAND       107       112         FT       STRAND       114       122         FT       STRAND       114       122         FT       STRAND       114       122         FT       HELIX       119       136         FT       HELIX       119       136         FT       HELIX       103       175         FT       STRAND       173       175         FT       HELIX       200       205         FT       HELIX       200       205         FT       HELIX       200       204         FT       HELIX       204       204         Storestore       Storestore       Storestore       Storestore         Storestore       Storestore       Storestore <td></td> <td></td> <td></td> <td></td> <td></td>					
PT         BELIZ         06         96           PT         STRAND         107         112           PT         STRAND         114         122           PT         MELIZ         129         166           PT         MELIZ         141         160           PT         MELIZ         170         172           PT         MELIZ         170         172           PT         STRAND         173         175           PT         MELIZ         204         205           PT         HELIZ         264         273           PT         HELIZ         266         290           PT         HELIZ         303         307           STRAND         SORDALAND         SORDALAND         SORDALAND           SORDALAND					
TT       STRAND       107       112         TT       STRAND       107       112         TT       STRAND       114       122         TT       STRAND       114       122         TT       STRAND       114       122         TT       STRAND       114       122         TT       MELIX       129       136         TT       STRAND       101       103         TT       STRAND       101       103         FT       STRAND       103       103         FT       HELIX       200       201         FT       HELIX       250       203         FT       HELIX       206       290         FT       HELIX       303       307         Starter       Starter       40620       Ntr, Sprater         Starter       Starter       40620	FT	HELIX	77	82	
TT STRAND       114       122         TT HELLIS       129       136         TT HELLIS       141       160         TT HELLIS       170       172         TT STRAND       173       175         TT HELIX       203       201         TT HELIX       244       253         TT HELIX       264       273         TT HELIX       264       273         TT HELIX       264       298         TT HELIX       363       367         SEQUENCE       351. AUG OF MERINE MENDADATAR LOOPERINT. OTOSPORYME.         VENETOONTAK       MANDIENTILAWAN MENDADATAR PFLANEERS FRANKULAWAN         VENETOONTAK       MANDIENTILAWAN LUMONT VILLOWANT MENDADATAR PFLANEERS FRANKULAWAN         VENETOONTH HELINGANG TATLC OTPERLAPHEN LEADATAR PFLANEERS FRENEMELLINGONT       THELINGONT	FT	HELIX	86	96	
TT       STRAND       114       122         TT       HELLIS       129       136         FT       HELLIS       141       160         FT       HELLIS       170       172         TT       STEAMD       173       175         TT       STEAMD       173       175         TT       STEAMD       120       201         TT       STEAMD       123       175         TH       LIS       202       203         TT       HELLIS       202       204         TT       HELLIS       244       253         FT       HELLIS       264       273         FT       HELLIS       264       273         FT       HELLIS       264       273         FT       HELLIS       264       273         FT       HELLIS       264       298         FT       HELLIS       303       306         HELLIS       264       298       174         HELLIS       303       306       189         SEGUALATEK       SEGUALATIONATION SEGUENCERS       FEMBELLIS         SEGUALATEK       SEGUENCERS       SEGUENCE	FT	STRAND	107	112	
FT       HELIX       129       136         FT       HELIX       141       160         FT       HELIX       170       172         FT       STRAND       131       160         FT       STRAND       131       160         FT       STRAND       131       163         FT       HELIX       203       205         FT       HELIX       200       211         FT       HELIX       244       253         FT       HELIX       264       273         FT       HELIX       264       273         FT       HELIX       204       263         FT       HELIX       264       273         FT       HELIX       204       263         FT       HELIX       204       273         FT       HELIX       204       273         FT       HELIX       205       209         FT       HELIX       346       348         SO       SOUTENCE       351       A1       40630         SO       SOUTENCE       STAL       A0640       HULINCONT         VIMHETONHY       MENUPOGONT </td <td></td> <td></td> <td></td> <td></td> <td></td>					
FT       HELIX       141       160         FT       HELIX       170       172         FT       STRAND       173       175         FT       STRAND       163       163         FT       HELIX       203       205         FT       HELIX       203       205         FT       HELIX       203       204         FT       HELIX       204       214         FT       HELIX       264       273         FT       HELIX       266       269         FT       HELIX       206       293         FT       HELIX       303       307         SEQUENCE       S31. Al-4       MENDARY       SEQUENCE         SEQUENCE       S31. Al-7       MENDARY       MENDARY         VENETORNY       MELIX       304       MENDARY         VENETORNY       MELIX       MENDARY       MENDARY         MENDARY       MENDARY       MENDARY       MENDARY         MENDARY       MENDARY       MENDARY       MENDARY         MENDARY       MENDARY       MENDARY       MENDARY         MENDARY       MENDARY       MENDARY       MENDARY					
PT         HELIX         170         172           TSTRAND         173         175           PT         STRAND         181         163           PT         HELIX         200         205           PT         HELIX         200         211           PT         HELIX         200         211           PT         HELIX         209         214           PT         HELIX         244         253           PT         HELIX         266         269           PT         HELIX         206         280           PT         HELIX         206         208           PT         HELIX         364         348           SO         SEQUENCE         351         AJ         4060           SO         SEQUENCE         351         AJ         4060           VKIMETONHY         MANLIDAGON         VLUTEYUMAKILINGON         HELIX				160	
TT       STRAND       173       175         TSTRAND       173       175         TSTRAND       181       103         FT       STRAND       181       103         FT       STRAND       181       103         FT       HELIX       203       205         FT       HELIX       219       234         FT       HELIX       219       234         FT       HELIX       244       73         FT       HELIX       256       269         FT       HELIX       236       293         FT       HELIX       303       307         SEQUENCIS       334       340       S0000227020EEEED CRC41         SEQUENCIS       334       340       S0000227020EEEED CRC41         MENADORY       SEQUENCIA       AFAMERY ENTREMENT REFERENCESS       SEQUENCIA         MENADORY       SEQUENCIA       AFAMERY ENTREMENT       SEQUENCIA         MENADORY       SEQUENCIA       AFAMERY ENTREMENT       SEQUENCIA         MENADORY       SEQUENCIA       SEQUENCIA       SEQUENCIA       SEQUENCIA         MENTROSONY       SEQUENCIA       SEQUENCIA       SEQUENCIA       SEQUENCIA				172	
PT     STRAND     101     103       PT     HELIX     203     205       PT     HELIX     209     211       PT     HELIX     219     234       PT     HELIX     244     253       PT     HELIX     266     269       PT     HELIX     206     293       PT     HELIX     206     294       PT	FT				
PT         HELIX         203         205           PT         HELIX         200         211           PT         HELIX         219         234           PT         HELIX         244         253           PT         HELIX         244         253           PT         HELIX         244         253           PT         HELIX         264         253           PT         HELIX         256         253           PT         HELIX         256         293           PT         HELIX         303         307           PT         HELIX         346         346           SEQUENCEX         SSLAMARKO         SEQUENCEX         ASAGENVERT           MERING         SAGENVERT         AGAGENVERT         AGAGENVERT					
PT       HELIX       200       211         PT       HELIX       219       234         PT       HELIX       244       253         PT       HELIX       244       253         PT       HELIX       266       269         PT       HELIX       256       290         PT       HELIX       266       290         PT       HELIX       303       300         00       SEQUENCE       351       A00         00       SEQUENCE       351       A000         NWINETONEY       MANILDRONV VELOVENTA       LOPERDIFIC         01       SEQUENCE       351       A000         02       SEQUENCE       MANILDRONV VELOVENTA       SOPODIZ27D2DEFESD CRC64;         NEWIRETONEY       MANILDRONV VELOVENTA       SOPODIZ27D2DEFESD CRC64;         VENETONEY       MELINDOOT       VUTDYOFAX ENVOR       SOPODIZ27D2DEFESD CRC64;         VENETONEY       HELIX       LANOPERINE, MANILDRONV       VELINA         VUTDYOFAX       EMANILDRONV       VELINDOOT       SOPODIZ         VUTDYOFAX       EMANILDRONT       UTTETINE, FORMULATING       SOPODIZ         ADOPIZITEK       USENETATION       HELINDOOT					
FT     HELIX     219     234       FT     HELIX     244     253       FT     HELIX     264     273       FT     HELIX     266     269       FT     HELIX     296     296       FT     HELIX     303     307       FT     HELIX     304     346       SQ     SQUENCE     351     A47       MONALANKS     SEQUENCEL     A56       WORMALANKS     SEQUENCEL     A164       VKIMETONINY     MILLDKORV     VENDERATION       MUTOFORMY     FILLANDERATION     FILLAND       MUTOFORMY     FILLANDERATION     FILLANDERATION       ADOPICIATION     HINDLANDERATION     FILLANDERATION       ADOPICIATION     HINDLANDERATION     HINDLANDERATION       ADOPICIATION     HINDLANDERATION     HINDLANDERATION       ADOPICIATION     HINDLANDERATION     HINDLANDERATION					
TT       HELIX       244       253         TT       HELIX       264       273         TT       HELIX       286       289         TT       HELIX       290       293         TT       HELIX       266       290         TT       HELIX       206       293         TT       HELIX       303       300         TT       HELIX       304       304         MONIALANCE       31       340       400         MONIALANCE       301       304       304         MONIALANCE       301       305       304         MONIALANCE       301       305       304         MONIALANCE       301       304       304         MONIALANCE       302       304       304         MONIALANCE       303       306       100         MONIALANCE       304       304       100         MONIALANCE       303       306       100         MONIALANCE       JALINOADI       100       100         MONIALANCE       JULINOADI       100       100         MONIALANCE       JULINOADI       100       100         MONIAL					
PT       TURN       264       273         PT       TURN       266       269         PT       HELIX       290       293         PT       HELIX       296       296         PT       HELIX       303       307         PT       HELIX       304       304         SQ       SQUENCE       351       A.F         MONALANKS       SEQUENCEL       ASE         MONALANKS       SEQUENCEL       ASE         MONALANKS       SEQUENCEL       ASE         MUNDALANKS       SEQUENCEL       MUNDALANKS         MUNDALANKS       SEQUENCEL       MUNDALA					
<pre>FT TURN 266 289 FT HELIX 296 293 FT HELIX 296 293 FT HELIX 303 307 FT HELIX 304 346 S000000 SEQUENCE S1: All FS SI All FS SODD22702DEEESD CBCG4: SEQUENCE S1: All FS SI All FS SODD22702DEEESD CBCG4: VENETORNY ANLIDEGRY VILLOUTENT NEEDILGANN PFLVELERS FINENELLING VENETORNY ANLIDEGRY VILLOUTENT NEEDILGANN PFLVELERS FINENELLING VENETORNY HELIDEGRE EMALEXALS.ULTETEINES FINENELLING NEUTOGAR HELIDEGRE EMALEXALS.ULTETEINES FINENELLING ADQUETER USGNUTENT SUSSANEEDILGANN PFLVELERS FINENELLING ADQUETER USGNUTENT SUSSANEEDILGANN PFLVELERS FINENELLING ADQUETER HILDEGRES FEMALEXALS.ULTETEINES FOR HELIDEGGY IGUTDFGFAR HEVGTTET HELIDEGRE HENDUCULTER FOR FORMULT ADQUETER USGNUTENT HENDUCULTER FOR FORMULT </pre>					
PT HELIX 290 293 PT HELIX 296 298 PT HELIX 303 307 PT HELIX 304 340 SOUTENCE 351 AJ, 40620 NW; 59DDD227D2DEEESD CPC64; NGNAAAKKS SEQEEVEREL AKAKEPFLKK WENPAGNTAH LÞOFERIKTL GTOSFGRVHL VKHMETONHY AMKILDROKV VKLOGIENTL NEKRILGANN PFPLVKLEFS FIXMENILINDV HKVYPGGANF SHLAPACAET HALAFKALDULTTETINS HKVYPGGANF SHLAPACAET HALAFKALDULTTETINS HKVYPGGANF SHLAPACAET HALAFKALDULTTETINS HKVPGGANF HKVPGANF HKVPGANF HKVPGGANF HKVPGANF HKVPGGANF HKVPGANF HKVPGGANF HKVPGANF HKVPGANF HKVPGANF HK					
FT HELIX 296 298 FT HELIX 303 307 FT HELIX 303 307 SEQUENCE 351 AA: 04620 HW; 59DDD227D2DEEESD CRC64; MORAAAARKO SEQUENTERL ARAEDFLAK VENFAQATAM LOOFENIETL GTOSPORVEL MORAAARKO SEQUENTERL ARAEDFLAK VENFAQATAM LOOFENIETL GTOSPORVEL MORADFUEL SISA MARAEDFLAKE VENFAQATAM LOOFENIETL GTOSPORVEL MORADFUEL HILSONGTUTL GTPERLARE, ILSNE THILDITTOLER FELLIDOODY IQUTDFORAR EVENGTUTL GTPERLARE, ILSNE THKAN DEWALOPLIY EMALOFPFF ADQ0101ETL VISANUTERU INSUE UNDERLARE PORLAREDFUEL MORADFUEL VISANUTERU INSUENCE HELIDOODY IDUTDFORAR EVENDET HISODOCHUM HELIDUTERUS FOR INSUENCE ADQ0101ETL VISANUTERUS INSUENCE FELLIDOOT					
PT HELIX 303 307 T HELIX 346 348 S0 SEQUENCE 351 AJ: 40620 HW; S9DDD227D2DEEESD CRC64; NGNAAAKKO SEQEEVENEL AKAKEDFLEK WENPAGNTAH LOOFERIKTL GTOSFGRVHL VKHETONHY AMKILDKOKY VKLOGIENTL NEKRILQANN PFPLVKLEFS FIKNMSKI/NYV HKYVPGGHT SHLPLACE THALFYLAR LLOAFT NFFLVKLEFS FIKNMSKI/NYV JCQTUTGTAK WYKGTVTLC GTPKTLAPEI LLSKYTMKAY DWUALQULY EMALGYPFF ADGPIGIFK IVSGMTVTLC GTPKTLAPEI LLSKYTMKAY DWUALQULY EMALGYPFF ADGPIGIFK IVSGMTVTLC GTPKTLAPEI LLSKYTMKAY DWUALQULY EMALGYPFF					
FT HELIX 346 348 SQ SEQUENCE 351 AJ: 40620 HW; S9DDD227D2DEEESD CRC64; MORAAAAKKO BEQEGVKEFL AKAKEDFLEK WENPAQNTAR LDQFERIKTL GTGBFGRVHL VERHETGRHT ARKILDROKV VELGUENTL NEKFLIQAVN PFFLVKLEFS FRAMSKLINV HEVYPGGAR HVRGTVTLC GTPETLAPET ILSKVINKAV DWWALOULIY ERAAGPPFF ADQF10ITER IVSGAVETPS HISDBACHGHT HNEVPLVLFK FFGLUKNOVA DIVISHKEVFAT					
30 SEQUENCE 351 AJ: 40620 HW. 59DD0227D2DEEESD CRC64: NGNAAAKKG SEQEEVERLA KARKEPLAKU KURPLANTAH LOOFERIKTI GTOSFORVHL VKHRETONHY AMKLIDKOKY VKLOCIENT. NEKRILGANN PFPLVKLEFS FIKNMSKI/HW HKYVPGORFF SHLPLACERE HHALFVLAL, ULTFFLING LOIFDELKF SHLLIDGOOY IQVTDF0FAK KVKOTVTLC GTPKTLAPEI ILSKYTMKAY DWUALOULIY EMALOFPFF ADGPIGIFK IVSGAVTF5 IISOSCHEME HHALFVLALF FORLIGKOVA IDVTDF0FAK KVKOTVTLC GTPKTLAPEI ILSKYTMKAY DWUALOULIY IMAACHPFFF					
NORAAAARKO BEGESVEEL ARAEBPLEK WENPAGNTAN LOOFENITU GTOSFORML VEHNETONHY ANKILDKONV VELKOIEHTL NEKELLONN PFLUKLEFS FKNNSNLYMV HEYPOGAK SHLADIGEL ENKLEFLOL DILTETILS LDLITEOLKE BELLIDGOOY IQVTDYGRAK RUKGITUTLC GTPETLAFEI ILSKYTNKAV DWALOULIY ERAKOFPFF ADOFICIEK UNGKYTFJ HISODENDEL HINDLYDLIK FKOLKDKON DINNEKVAT					MU: 59DDD227D2DFFF5D CBC64:
VKHRETCHHY ARKILDRORV VELOCIENTL NEKRILGANN PFFLVRLEFS FRANSRLIVAN NEVPGORTS SHLBALCASE ENALGEVAL OLITETEINE ELLINDOOT IQVTDFORAR SWORTVILG OTPETLAREI ILSKYTNKAV DUWALOULIY EHALOPPFF ADOFICIEN IVSKYTVETS HISODEMELM HMENDVDLIK FORLINDON	~¥				
REVPOSCHY SHL <u>PLACES ENALEVIAC, ULTY</u> TLES LD.IYEDLER ENL.DGOGY IQVTDYGFAR RVKGHTWILC GTPEYLAPEI ILSKYNKAV DWUALGVLIY ERALGYPPFF ADQFIGTER IVSGK <del>WTS ILSGERLEL FILL</del> VILLER FORLEGIND DIRHKWFAT					
IQVTDFGFAR RVRG <mark>H</mark> TWTLC GTPEYLAPEI ILSK <mark>D</mark> YNKAV DWWALGVLIY EHAAGYPPFF Adqpiqiyek ivsg <del>vrppp hposphyll rvll</del> yvdltk rfgnlwngvn diknhkwfat		VARIATIONI	T ARAILD	COPS VALL	VIERIE REFEICAVR FFEVALETO FAURALINV
ADQPIQIYEK IVSGRVFFPS HPSSPLRDLE NHLLVDLTK RFGNLKNGVN DIKNHKWFAT					
INARTING APALITUCE ALANDED IFFERENCE I					
*		TPATATION	N VEAPFI	FAIR OFOL	TONLAR TERPORTAL NEW AND LARGE L

According to Swissprot, both T1 and T3 are possible phosphorylation sites. If you really needed to know which was the case here, or whether it was a mixture, you'd have to acquire more data. Maybe try a different enzyme or target the incomplete cleavage peptide that includes the preceding KG so as to move the sites towards the centre of the peptide, where you might get stronger b and y fragments

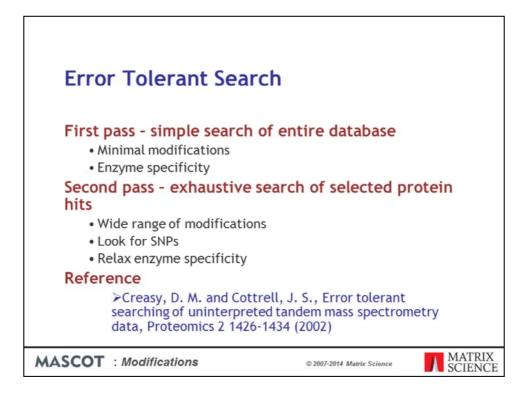


If you are using Mascot 2.3 or earlier, the delta score calculation is not performed in Peptide View. These are our suggested guidelines when using Mascot for site analysis:

If alternative sites differ by 20 in score, safe-ish to disregard lower one(s)

If alternative sites have similar scores, you may be able to choose one by inspection. But, be careful ... one peak is just one peak

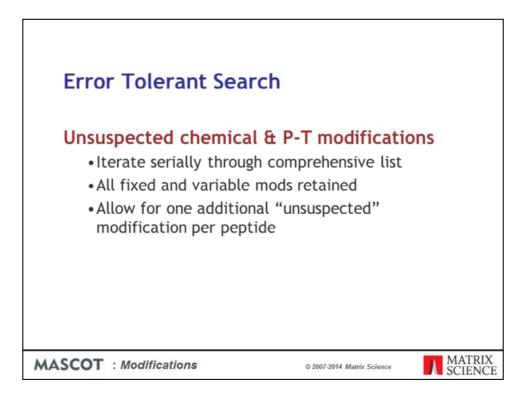
Often, you just can't differentiate between adjacent sites, even with great data.



Now, back to the challenge of finding PT modifications. There are many hundreds of modifications in Unimod, yet I've emphasised the importance of using the minimum number of variable modifications in a search. So, how are we supposed to find unusual modifications?

If you are searching uninterpreted MS/MS data, the efficient way to find unusual modifications, as well as variations in the primary sequence, is a two pass search. The first pass search is a simple search of the entire database with minimal modifications. The protein hits found in the first pass search are then selected for an exhaustive second pass search. During this second pass search, we can look for all possible modifications, sequence variants, and non-specific cleavage products.

Because only a handful of entries are being searched, search time is not an issue. It would be extremely difficult to calculate meaningful statistics for the additional matches in an error tolerant search, and we don't report expect values. The evidence for the presence of any particular protein are the matches from the first pass search. The additional matches from the second pass search serve to increase coverage and may discover interesting modifications or SNPs.

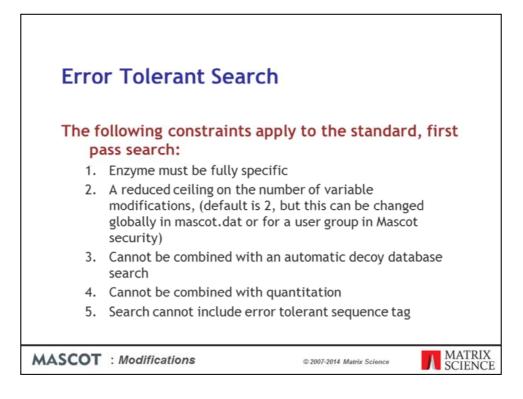


For modifications, an error tolerant search looks for one unsuspected modification per peptide in addition to those mods specified as fixed or variable. This is sufficient because it will be rare to get two unsuspected mods on a single peptide

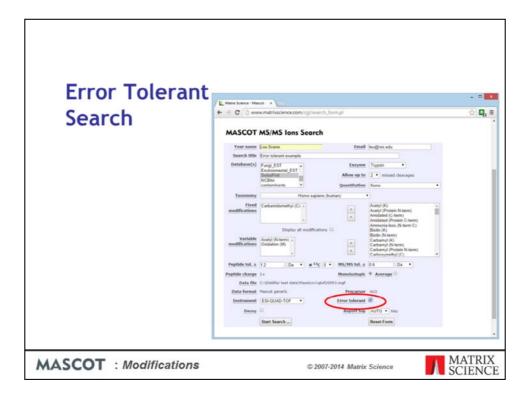
Error Tolerant Search		
Primary sequence variants		
<ul> <li>Protein database</li> </ul>		
Look for all residue substitu	utions	
No attempt to identify sing deletions because of frame		ns &
<ul> <li>Nucleic acid database</li> </ul>		
Look for all single base subs & deletions	stitutions, inser	rtions
MASCOT : Modifications	© 2007-2014 Matrix Science	MATRIX SCIENCE

The error tolerant search also looks for sequence variants, such as single nucleotide polymorphisms (SNPs) or sequencing errors.

For a protein database, we can't look for the consequences of inserted or deleted bases, because these give rise to frame shifts, and the entire sequence changes from that point on.



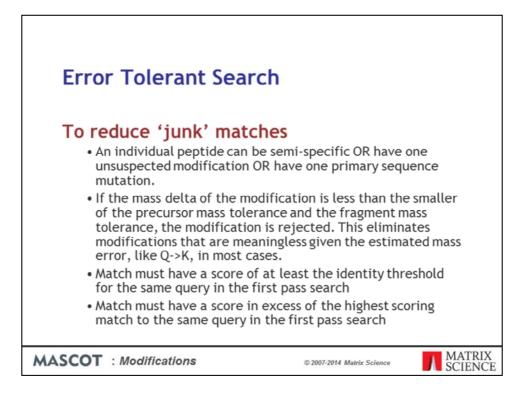
There are some constraints on the standard, first pass search



Otherwise, submitting the search is just like submitting a standard search except that you check the Error Tolerant Checkbox

Select	AR	Select None			Error (			_%2Fdat			915%2FFTgcfieOLdat
Select	rue	Select North	oearca	Selected	- Liroi (	orer					
	P81_H		s: 58259	Score: 519				Sequenc		10(9)	
				stal type OS			Otheratio	PP PE+1 5	V*2		
	neck	to include	this hit in	error tole	rant sear	cn					
	very	Observed	Hr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
1	27	462.6807	923.3468	923.5116	-0.1649	0	33	0.17	1		R. FPYVALSK. T
	41	517.1760	1032.3375	1032.5604	-0.2229	0	70	4e-05	2		R.GSSIFGLAPGR.A
8	52	545.6819	1089.3491	1089.5819	-0.2327	0	(53)		1		<pre>B.G551FGLAPGR.A + [+57.0215 at 52]</pre>
2	62	564,6804	1127,3463	1127.5764	-0.2301	0	10	32	1		R.GFFLFVEGGR.I
×	12		1133.2987		-0.2511	0	44	0.011	1		R.GNEVISVMNR.A + Oxidation (N)
8	46		1226.3856		-0.2473	0	27	0.56	1	U	K.LGPEIPLAUDR.F + Oxidation (M)
8	102		1304.4057		-0.2780	0	(#7)	5.8e-07	1		K. CNFQTIGLSAAAR. F
8	124		1418.4324		-0.2942 -0.3011		95 73	1.20-05	1		K_GNFQTIGLSAAAR.F = [ <u>+114.0429</u> at N-term G] R.NHYSDADNPASAR.Q
2	133		1494.3828		-0.2866		88	1.26.03			L.DPSLHEHTEAALR.L + 2 Owidation (M)
8	136		1507.3582		-0.3109		(44)		-		R_NEYSDADVPASAR.Q + [+57.0215 at N-term N]
ŝ	145			1575,7814	-0.3418		(61)		-		R.ALTETINEDDALER.A + [-48.0000 at F8]
8	156				-0.3343		106	6.2e-09	-		R.ALTETIHFDDAIER.A + Oxidation (M)
8	165		1680,4474		-0.3554	8	(75)		1		R_ALTETINFODALER.A + Oxidation (M); [+41.0266 at N-term A]
2	170	864,2888	1726.5629	1726.9294	-0.3664	0	44	0.0092	1		K.AYTVLLYGNGPGYVLK.D
8	175	586.4951	1756.4635	1756.8420	-0.3786	0	(45)		1		G. 11PVEEENPOFUNR. E
10	176	879.2425	1756,4705	1756.8420	-0.3715	0	83		1		G. TEPVEEENPORING. E
8	179	593.4834	1777.4285	1777.7764	-0.3478	0	45		1		K.HVPDSGATATAYLCGVE.G + [+31,9357 at C-term K]
8	204	956.2437	1910.4729	1910.8601	-0.3872	0	30	0.23	1		R.OSTLDPSLMEMTEAALR.L + 2 Oxidation (M)
×	205		1949.6055	1950.0245	-0.4190	0	85	6.5e-07	1		K.MLIIFLGDGBGVSTVTAAR.I = Oxidation (M)
×	202		1950.4534		-0.4021	0	(27)	0.41	1		K.DGARPOVTESESGSPEYR.Q
8	211		1965.5039		0.6327	0	(72)		1		K.DGARPOVIESESGSPEYR.Q = [+14.0157 at TH]
8	213		1990.6336		-0.4174	0	(58)		1		<pre>W_NLIIFLGDGBGVSTVTAAR.1 + Oxidation (M); [+41.0266 at N-term N]</pre>
8 8	216	1001.2027	2000,3908	2000.8058	-0.4150	0	(67)	4.1e-05 4.9e-06	1	U	R.MGTPDPEYPDDYSQGGTR.L + Oxidation (M)
2	217		2000.3919		-0.4139	-	76	4.76.00	1	0	R.HGTPOPEYPDOYSQGGTR.L = Oxidation (M) K_DGARPOVTESESGSPEYR.Q = Acetyl (N-term); [=15,0109 at N-term 0]
2	222		2007,4400		0.6073		(61)			U	R_DGARPOVISESGAPETR.Q + Acetyl (N-term); [-12.0109 at N-term 0] R_MGTPDPEYPDOVSQGGTR.L + Acetyl (N-term); Oxidation (N); [-0.9540 at 67]
ŝ	224		2057.4016		-0.4256		(45)		-		R_MGTPDPEYPDDYSQGGTR.L + Oxidation (M); [+57,0215 at N-term M]
8	227		2131.7013		-0,4327	1	16	4.9	-	U	K.LGPEIPLAYDRFPVVALSK.T + Oxidation (M)
2	252		2350,6103		-0.4927		(69)		1	U	R_QOSAVFLDEETHAGEDVAVFAR_G + [-17,0265 at N-term 0]
8	253		2367,6341		-0.4954	0	94	7.4e-08	1	U	R.QQ5AVFLDEETHAGEDVAVFAR.G
8	260	809.2208	2424.6406	2425,1510	-0.5104		(66)		1	U	R_QQ5AVFLDEETHAGEDWAVFAR_G + [+57,0215 at N-term Q]
1	274	914,9160	2741.7263	2741.2306	0.4956		(41)		1		R.QEGCQDIATQLISMEDIDVILGGGR.K + Oxidation (N); [-79,9568 at C4]
ø	275	978 5878	2758 2415	3750 3583	-8 6167		- 64				# OFFCCONTATION TRANSPORTED FOR A Averal (Notema): Ovidation (N): 1-0 0476 at F21

And here is the first hit of the results report. The additional matches, found in the error tolerant search, are the ones without expect values. One of these, query 133, is a simple, non-specific peptide with a very good score. There's another example for query 176. The error tolerant search is a much better way of picking up non-specific peptides than searching the entire database with semi-trypsin or no enzyme. We only fail to get such matches in an error tolerant search if there are no matches to the protein in the first pass search. However, you have to ask yourself whether you would believe a protein hit in which the only peptide match was non-specific. I think the answer is no.



The matches from an error tolerant search are aggressively filtered to remove junk matches

Select	Al	Select Norw	e Search	Selected	Error t	olera	nt				o stoleti 🖌 o kuoletti		6
1		ne phosphat		Score: 519 stal type 05- error tole	+Homo sapl	lens		Sequence P PE+1 S		0(9)			
	very	Observed	Mr(expt)	Mr(calc)	Delta #	tiss	Score	Expect	Rank	Unique	Peptide		
8	22	462.6807	923.3468		-0.1649		33	0.17	1		R.FPYVALSK.T		
	41	517.1760	1032.3375		-0.2229	0	70	4e-05	2		R.GSSIFGLAPGK.A		
×	53	545,6819	1089.3491		-0.2327		(53)		1		R.0551FGLAPGK.A = [ <u>+57.0215</u> at 52]		
8	52	564.6804	1127.3463	1127.5764	-0.2301	•	10	32	1		R.GFFLFVEGGR.1		
8	65		1133,2987		-0.2511	•	44	0.011	1		R.GNEVISVENR.A + Oxidation (M)		
8	.85	614.2001	1226.3856		-0.2473		27	0.56	1	U	K.LGPEIPLAEDR.F = Oxidation (M)		
8.8	100	653.2101	1304.4057	1304.6837	-0.2780	2	(87)	5.8e-07	1		K. GNEQTICLEARAR.F		
ŝ	126	726.1806		1418.7266	-0.3011			1.20-05			K_GNFQTIGLSAAAR.F + [ <u>-114.0429</u> at N-term G] R.NHYSDADVPASAR.Q		
ŝ.	133		1494.3828		-0.2866						L.DPSLMEMTEAALR.L + 2 Oxidation (M)		
ŝ.	136		1507.3582		-0.3109	0	(44)				R_MMYSDADVPASAR.Q + [+57.0215 at N-term N]		
ã	145				-0.3418		(61)		1		R.ALTETINEDDAIER.A + [-48.C D at F8]		
8	156	820,7283	1639,4420	1639,7763	-0.3343	0	106	6.20-05	1		R.ALTETIBFDOALER.A + Oxidat		
8	165	841.2310	1680,4474	1680.8029	-0.3554		(75)		1		R_ALTETIMFDDAIER.A + Oxidat Possible Assignments:		
2	170	864.2888	1726,5629	1726.9294	-0.3664	0	44	0.0092	1		K.AYTVLLYGNGPGYVLK.D Carbamidomethyl (N-term) [+57.0215]		
æ	175	586.4951	1756,4635	1756.8420	-0.3786	0	(48)		1		G.IIPVEEENPDFWNR.E Carboxymethyl (N-term) [+57.0215]		
8	176	879,2425	1756.4705	1756.8420	-0.3715	0	63		1		G.IIPVEEENPOFUNR.E Delts:H(6)C(3)O(1) (Protein N-term) [+58.0419]		
×	179	593,4834	1777.4285	1777.7764	-0.3478	0	45		1		K.HVPDSGATATAYLCGVK.G + [+3		
×	204	956.2437	1910,4729	1910,8601	-0.3872	0	30	0.23	1	U	R.OSTLDPSLMEMTEAALR.L + 2 Oxidation (M)		
8	205	975,8100	1949.6055	1950.0245	-0.4190		85	6.5e-07	1		K.NLIIFLGDG%GVSTVIAAR.1 + Oxidation (M)		
ø	202	976.2340	1950.4534	1950.8555	-0.4021		(27)	0.41	1		K.DGARPOVTESESGSPEYR.Q		
8	211	656.1752	1965,5039	1964.8712	0.6327		(72)		1		K.DGARPDV <u>T</u> ESESGSPEYR.Q + [ <u>+14.0157</u> at T8]		
8	213	664.5518	1990.6336		-0.4174	0	(58)		1		K_NLIIFLGDGMGVSTVTAAR.I + Oxidation (M); [+41.0266 at N-term N]		
8	216	1001.2027	2000.3908	2000.8058	-0.4150	•		4.1e-05	1	U	R.MGTPDPEYPDDYSQGGTR.L + Oxidation (M)		
8	217	667.8046	2000.3919	2000.8058	-0.4139	0	76	4.9e-06	1	U	R.BGTPDPEYPDDVSQGGTR.L + Oxidation (M)		
8	218	670.1561	2007.4466	2007.8770	-0.4304		75		1		<pre>K_DGARPOVTESESGSPEYR.Q + Acetyl (N-term); [+15,0109 at N-term D]</pre>		
8	222	681.8205	2042.4397	2041.8324	0.6073	•	(61)		1	U	<pre>#_HGTPOPEYPODVSQGGTR.L + Acetyl (N-term); Oxidation (H); [-0.9640 at E7]</pre>		
8	224	1029.7081	2057.4016	2057.8273	-0.4256		(45)		1	U	R_HGTPDPEYPDDVSQGGTR.L + Oxidation (M); [+57.0215 at N-term M]		
8.8	227	711.5744	2131.7013		+0.4327	1	16	4.9	1	0	K.LGPEIPLAUDRFPYVALSK.T + Oxidation (M)		
8.8	252	784.5440	2350.6103		-0.4927		(69)	7.44-08	1	U	R_QQSAVPLDEETHAGEDVAVFAR.G + [-17,9265 at N-term Q]		
8.8	253	190,2187	2367,6341 2424,6406	2368.1295	-0.4954		(66)	7.48-08	1		R. QQSAVPLDEETHAGEDVAVFAR.G		
8	250		2741.7263		0.4956		(41)		1		R_QQSAVPLDEETHAGEDVAVFAR.G + [=57.0215 at N-term Q] R.QEGCQDIATQLISNEDIDVILGGGR.K + Oxidation (N); [=79.9568 at C4]		
a.	1.70	030.0030	5370 3447	3350.3503	0.4000	-	(41)				R OPECODITION TOWNTOCION R + DESTIN (N); [172,2200 BC (4] R OPECODITION TOWNTOCION R + DESTIN (N-term): Oridation (N); [-0.9476 at F	1	
script	veid(5)					1			÷			1	

Take a look at the match to query 136. The mass tolerance for this search was fairly wide, so the observed mass difference could correspond to either carbamidomethylation or carboxymethylation at the N-terminus. Since this sample was alkylated with iodoacetamide, we would choose carbamidomethylation as the more likely suspect, especially as this brings the error on the precursor mass into line with the general trend, whereas carboxymethylation would give an error of +0.6 Da. The assignment to carbamidomethylation is also very believable, because this is a known artefact of over-alkylation. The same modification can be seen in this screen shot for three other queries

	C	🗈 www.m	atrixscience	.com/cgi/m	aster_res	ults.p	l?file-	%2Fdat	1%2F	201409	15%2FFTgcfieOL.dat	-	Q,
	65	567.6567	1133.2987	1133.5499	-0.2511	0	44	0.011	1		B.GNEVISVENR.A + Oxidation (H)		-0
2	85	614,2001	1226.3856	1226.6329	-0.2473		27	0.56	1	U	K.LGPEIPLANDR.F = Oxidation (M)		
2	100	653.2101	1304,4057	1304.6837	-0.2780	0	(87)	5.8e-07	1		K. CNFQTIGLSAAAR. F		
2	124	710.2235	1418,4324	1418.7266	-0.2942		95		1		K_CNFQTIGLSAAAR.F - [-114.0429 at N-term G]		
8	126	726.1806	1450,3465	1450.6477	-0.3011	.0	73	1.2e-05	1		R. NHYSDADVPASAR.Q		
2	123	499.1349	1494.3828	1494.6694	-0.2866	0			1		L.DPSLMEMTEAALR.L + 2 Omidation (R)		
2	126	754.6864	1507.3582	1507.6691	-0.3109		(44)		1		R_NWYSDADVPASAR.Q = [+57,0215 at N-term N]		
- 2	145	526.1538	1575.4396	1575,7814	-0.3418	٠	(61)		1		R.ALTETINEDDAIER.A + [_48.0000 at F8]		
2	156	820.7283	1639,4420	1639.7763	-0.3343	0	106	6.2e-09	1		R.ALTETIMFODAIER.A + Oxidation (M)		
- 92	165	841.2310	1680,4474	1680,8029	-0.3554	0	(75)		1		R_ALTETIMFDDAIER.A + Oxidation (M); [+41.0266 at N-term A]		
1	170	864.2888	1726.5629	1726.9294	-0.3664	0	44	0.0092	1		K.AYTVLLYGNGPGYVLK.D		
18	175	586,4951	1756,4635	1756,8420	-0,3766	0	(48)		1		G. IIPVEEENPDFUMR. E		
æ	176	879.2425	1756.4705	1756.8420	-0.3715	0	83		1		G. LIPVELENPOPUNE.E		
8		593,4834	1777.4285	1777.7764	-0.3478		45		1		K.HVPDSGATATAYLCOVE.G + [=31.5352 at C-term K]		
2	204	956.2437	1910.4729	1910.8601	-0.3872	0	30	0.23	1	U	R.OSTLDPSLMEMTEAALR.L + 2 Oxidation (M)		
R		975.8100	1949,6055	1950.0245	-0.4190		85	6.5e-07	1		K.NLIIFLODG@GVSTVTAAR.I = Oxidation (M)		
18		976.2340	1950.4534	1950.8555	-0.4021	0	(27)	0.41	1		K. OGARPENTESESGSPEVR. Q		
2		656,1752	1965.5039	1964.8712	0.6327		(72)		1		K.DGARPOVIESESGSPEYR.Q + [114.0157 at T8]		
R		664.5518	1990.6336	1991.0510	-0.4174	0	(58)		1		K_NEIIFLGDGBGVSTVTAAR.I + Oxidation (M); [+41.0266 at N-term N]		
8	215	1001.2027	2000.3908	2000.8058	-0.4150		(67)	4.10-05	1	U	R. MGTPDPEYPDDYSQGGTR.L + Oxidation (M)		
R		667.8046	2000.3919	2000.8058	-0.4139	0	76	4,9e-06	1	U	R.BGTPDPEYPDDYSQGGTR.L + Oxidation (M)		
×	218	670.1561	2007,4466	2007.8770	-0.4304		75				<pre>K_DGARPOVIESESGSPETR.Q + Acetyl (N-term); [+15,0109 at N-term 0]</pre>		
2	222	681.8205	2042,4397	2041.8324	0.6073	0	(61)		1	U	R_MGTPDPEVPDDYSQGGTR.L + Acetyl (N-term); Oxidation (M); [:0.2840 at E7]		
×		1029,7081	2057,4016	2057,8273	-0.4256	0	(45)		1	U	R_MGIPOPEYPDOYSQGGIR.L + Oxidation (M); [ <u>+57,0215</u> at N-term M]		
2	227	711.5744	2131.7013	2132.1340	-0.4327	1	1.6	4.9	1	U	K.LGPEIPLAMORFPYVALSK.T + Oxidation (M)		
18		784.5440	2350.6103	2351.1030	-0.4927	0	(69)		1	0	R_QQSAVFLDEETHAGEDVAVEAR.G + [-17.0265 at N-term Q]		
×	253	790.2187	2367.6341	2368.1295	-0.4954	0	94	7.4e-08	1	U	R.QQSAVPLDEETHAGEDVAVFAR.G		
8			2424.6406		-0.5104	0	(66)		1	U	R_QQSAVFLDEETHAGEDVAVFAR.G + [+57.03		
2			2741.7263		0.4956	0	(41)		1		R.QEGEQDIATQLISMEDIDVILGGGR.K + Owid Possible Assignments:		
8.8			2758.7415 3232.8763		-0.6167 -0.6867		90	16	1		R_QECCQUARQLISHEDDVILGGR.K + Acet B.AGQLISEEDTLSLVTADHSHWISEGGYPLB.G	[2]	
8	281 PPRILE Alkeli	1078.6327	3232.8763 ss: 57626 tase, placer		-0.6867 Match	es: 2	10	Sequence	F: 15(	(#)		[2]	
	Query 22	Observed 462.6807	Mr(expt) 923.3468	Mr(calc) 923.5116	Delta -0.1649	0	33	0.17	1	Unique	Peptide R.FPYVALSK.T		
	41				-0.2229	0	70	48-05	2		R.OSSIFOLAPOK.A		
	52	245,6819	1087,3491	1089.5819	+0.2327	.0	(53)	1.1	1		R.0551F0LAPGK.A + [+57.0215 at 52]		
nstig	diversit(0)					1							
		-							_			ATF IEN	-

Another easily believable assignment is pyro-Glu for the match to query 252.

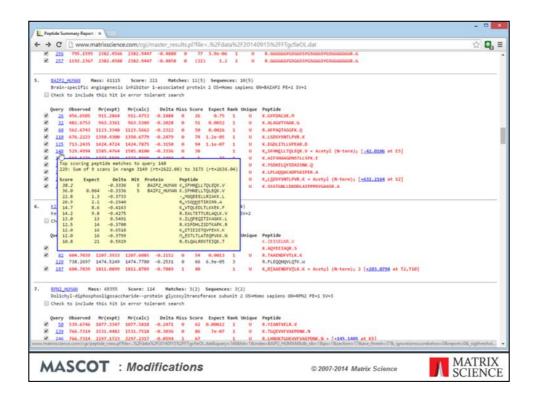
<pre>e d: StorAdd 113.3e9 113.3e9 123.4e9 -0.311 0 44 0.011 1 0.0007598.4 - 0.0141500 ()</pre>		C	www.m	atrixscience	e.com/cgi/m	aster_resi	ults.p	?file=	_%2Fdat	1%2F	201409	915%2FFTgcfieOL.dat 🔂 🛃 🛙
<pre>P 120 45.200 13.00.407 130.407 120.200.20 10 (07) 5.0-07 1 t. CONTINUESSAULT. # 151.5222 4t %*term 6] 121 497.2014 145.200 - 0.2014 0 (07) 1.2.001 1 (07) 121 497.100 145.100 145.100 10 (07) 121 497.100 145.100 145.100 10 (07) 121 497.100 145.100 145.100 10 (07) 121 497.100 145.100 145.100 10 (07) 121 497.100 145.100 145.100 145.100 10 (07) 121 497.100 145.100</pre>	1	65	567.6567	1133.2987	1133.5499	-0.2511	0	44	0.011	1		R.GNEVISV9NR.A + Oxidation (H)
Q       12       210.225 148.022 448.024 448.720       -0.242 0       0       0       1       export[TLELAMA.F1 - 2 Oxidation (T)         Q       12       210.110       140.720       150.7401       0       12.1200       150.7401       0       12.1200         Q       12       210.110       150.7401       0       0       1       1.07410000000000000000000000000000000000	2		614.2001	1226.3856	1226.6329	-0.2473		27	0.56	1	U	K.LGPEIPLAUDR.F - Oxidation (M)
Q       112       09:100       14:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:00:00:00       12:0		100	653.2101	1304,4057	1304.6837	-0.2780	0	(87)	5.8e-07	1		K. CNEQTICLEANAR. F
Q       11       09.140       140       54.040       15.		124	710.2235	1418,4324	1418.7266	-0.2942		95		1		K_GNFQTIGLSAAAR.F - [-114.0529 at N-term G]
Q       15       556.4664       1087.582       1557.584       -0.3100       0       (44)       1       B.ANTINGROMSSAD.47       (557.681)       -0.500       15         Q       155       1557.584       557.584       -0.5148       0       06       0.200       1       B.ANTINGROMSSAD.47       -0.5200 eff 61         Q       155       412.5       1557.584       -0.5554       0       64       0.002       1       B.ANTINGROMSSAD.47       -0.644cine (70)       [s1.0256] eff 8-term A]         Q       125       557.584       -0.5554       0       44       0.002       1       B.ANTINGROMSSAD.47       -0.644cine (70)       [s1.0256] eff 8-term A]         Q       125       557.4705       1757.474       -0.5756       0       3       1       -11PERTIFERENTIAL - 0.064cine (70)       [s1.0252] eff C-term E]         Q       125       575.4705       1559.4707       -0.4707       0       80       0.33       1       0       1.571444555       1.570.4714       1.57144       0.5114741000007VTAAL : -0.51445100(0)       0.5714741000007VTAAL : -0.51445100(0)       0       0.57147400007VTAAL : -0.51445100(0)       0       0.57147400007VTAAL : -0.51445100(0)       0       0.57147400007VTAAL : -0.5144510000000       0       0.511							0		1.2e-05	1		
<pre>8 135 325-1354 1357.439 1357.734 4.5.348 0 (61) 1 1 8.ATTINEDALTS.4 [-1, 12, 0020 et f8] 135 487.738 1507.430 1507.734 -0.3480 0 (65 0.2-09 1 8.ATTINEDALTS.4 = Oxidation (7) 14 125 544.7318 1507.436 1367.438 -0.3556 0 (75) 1 8.ATTINEDALTS.4 = Oxidation (7) 15 125 544.731 1356.438 1757.546 -0.3568 0 (44) 1 0 A.TIVETINEDALTS.4 = Oxidation (7) 15 12 1354.438 1757.546 -0.3568 0 (44) 1 0 A.TIVETINEDALTS.4 = Oxidation (7) 15 12 1354.438 1757.546 -0.357 0 (44) 1 0 A.DIVETINEDALTS.4 = Oxidation (7) 15 22 1354.438 1757.546 -0.357 0 (44) 1 0 A.DIVETINEDALTS.4 = Oxidation (7) 15 22 1354.438 177.746 -0.347 0 (48) 1 1 A.TIVETINEDALTS.4 = Oxidation (7) 15 22 1354.438 177.746 -0.347 0 (48) 0 .2 1 0 B.DITUTINEDALTS.4 = Oxidation (7) 15 22 1354.438 177.746 -0.347 0 (48) 0 .2 1 0 B.DITUTINEDALTS.4 = Oxidation (7) 15 22 1354.438 177.746 -0.347 0 (48) 0 .2 1 0 B.DITUTINEDALTS.4 = Oxidation (7) 15 22 1354.438 177.746 -0.347 0 (48) 0 .2 1 0 B.DITUTINEDALTS.4 = Oxidation (7) 15 22 1354.438 177.746 -0.347 0 (48) 0 .2 1 0 B.DITUTINEDALTS.4 = Oxidation (7) 15 22 1354.438 177.746 -0.347 0 (20) 8 4 5.5.407 1 E B.DITUTINEDALTS.4 = Oxidation (7) 15 22 1350.431 1340.355 0 -0.4114 0 (27) 8.4 1 E.COMPUTINEDALTS.4 = Oxidation (7) 15 21 1606.4558 1906.053 1964.4712 0 0.577 0 (20) 1 E B.DITUTUTINEDALTS.4 = Oxidation (7) 15 21 667.558 0 00.359 2000.0008 -0.4124 0 (58) 1 E E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.E.E</pre>		123	499.1349	1494.3828	1494.6694	-0.2866	0			1		L.DPSLEEMTEAALR.L + 2 Omidation (R)
0       15       482-7283 1609-4420 1509-7783 -0.3143 0       100 6.02-09 1       R_ATTINEDROMERAL + Outdation (N)         0       155       442-208 1720-5620 1726-529 1726-529 -0.3064 0       44 0.0002 1       R_ATTINEDROMERAL + Outdation (N) [sil_0266 st N=term A]         0       125       586-7497 1726-5620 1726-5429 -0.3064 0       44 0.0002 1       R_ATTINEDROMERAL + Outdation (N) [sil_0266 st N=term A]         0       125       586-7497 1726-5620 1726-5440 0.0.3175 0       68 3       1       6.11PETINEDROMERAL + 2 Outdation (N)         0       125       586-7497 1596-6780 1766-6440 0.0.3175 0       68 5.5=07 1       1.05115F149810700.41. + 2 Outdation (N)         0       120 1956-6780 1950-6030 1950-6337 0       6172 0.41 1       1.05115F149810700.41. + 2 Outdation (N)         0       121 006-1735 1956-6780 1956-6335 0.950 -0.4337 0       6172 0.41 1       1.05115F1498107000420717A8.1 + 0.0144 1       1.011671010000717A8.1 + 0.0144 1         0       121 006-1735 1956-6780 1956-6335 0.900-079 - 0.4139 0       7.5 4.990 1       1.001671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.0144 1       1.011671000000718.4 + 0.01144 1       1.011671000000718.4 + 0.0116710000000718.4 +										1		
0       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       641.210       120       120       120       120       120       120       120       120       120       120       120       120       120       120       120       120       120       120       121       120							٠					
0       122       564.2885       1725.529 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>0</td> <td></td> <td>6.2e-09</td> <td>1</td> <td></td> <td></td>							0		6.2e-09	1		
0       122       595.4053       175.4439       575.4439       6.1197ETSMORDANE.E         0       125       577.4245       175.4439       777.424       0.5175       6       5       1       F.INTESMORDANE.E         0       125       577.4245       175.4409       177.7424       0.5175       6       5       1       F.INTESMORDANE.E         0       125       577.4500       1596.4534       1607.4107       1597.414       1608.111       161.4026.418.15.1197.416.4107       1596.414       158.456777777777777777777777777777777777777							0			1		
0       125       979-245       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1759-2490       1500-2492       1510-2							0		0.0092	1		
0       12       99.4844       177.488       178.487       17										1		
0       221       055.247       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0479       198.0455       198.0455       198.0455       198.0455       100.017       100.017       100.0000000000000000000000000000000000							0			1		
<pre>2 22 07.100 199.003 190.0245 -0.419 0 0 3 0.57-07 1 K.NIFFLOODDNYTFAR.1 - 0.414tim (N) 2 22 07.2146 190.0255 07.0245 -0.413 0 (2) 2 21 100.1751 090.031 191.0310 -0.4137 0 (2) 2 1 100.1751 090.031 191.0310 -0.4137 0 (2) 4 11 00.1751 090.031 191.0310 -0.4137 0 (2) 4 11 00.1751 090.031 191.0310 -0.4137 0 (2) 4 11 00.1751 090.031 191.0310 -0.4137 0 (2) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 11 00.1751 090.031 191.0310 -0.4137 0 (5) 4 12 00.1751 090.031 190.0418 -0.4139 0 (5) 4 11 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.1751 090.0181 -0.4140 0 (5) 4 10 00.17</pre>							•	-		1		
0       202       979-2340       959-4534 <td></td> <td>_</td> <td></td> <td></td> <td></td> <td></td> <td>•</td> <td></td> <td></td> <td>1</td> <td>0</td> <td></td>		_					•			1	0	
0       211       006.1752       1980.1009       1990.												
0       211       665-518       1990.6336       1991.635       0       643       1       w.R.11FL00000571VPV0075005R.1.+ 0.0164       Postble Assignments:         0       212       667.8065       2000.0395       2000.0985       -0.4159       0       8.0017097V0007505R.1.+ 0.0164       Postble Assignments:         0       212       667.8065       2000.0935       2000.0935       0.6159       0       8.0017097V0007505R.1.+ 0.0164       Postble Assignments:       m.0)         0       212       677.805       2007.0970       -0.6155       0       6.0157       0       8.0017097V0007505R.1.+ 0.0164       Postble Assignments:       m.0)         0       222       677.805       2007.0970       -0.6155       0       6.015       0       8.0017097V0007505R.1.+ 0.0164       Postble Assignments:       m.0)       .5590 at C7]       1.590 at C7]       1.560 at C7]       1.574 at C7]       1.517 at C7]       1.590 at C7]       1.590 at C7]       1.560 at C7]       1.574 at C7]       1.517 at C7]       1.520 at C7]       1.517 at C7]       1.520 at C7]       1.517 at C7]       1.520 at C7]       1.520 at C7]       1.517 at C7]       1.520 at C7]       1.520 at C7]       1.520 at C7]       1.520 at									0.41	1		
0       212       610.2077       2000.2080       2000.2083       0.4130       0       4.90100144000000000000000000000000000000												
Q       222       642.6865       2000.3913       2000.4935       00014       76       4.90-06       1       0       8.001709710705500578.1.0       0.1611       10.1614.057											1.0	
0       210       000.0000       2000.0000       000.00000       000.00000       000.00000       000.00000000000       000.000000 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>3</td><td></td><td></td></t<>										3		
0       222       001.8095       2002.4197       2001.		_							4,96-00		.0	
222       1027.0012       0027.0012												
0       222       711.5744       213.7083       213.108       -0.4327       1       15       4.8       1       0       K.LOPETPAQDEPTVALES, 4       Main []       [[12.2021]         0       232       782.5440       235.0180       -0.4027       1       15       4.8       1       0       K.LOPETPAQDEPTVALES, 4       [[12.2021]       15       12       Registration = state							0			2		
0       212       788.5440       2350.6190       2351.1000       -0.6927       0       69)       1       0       R.QQSLAVIDETIMECEDVARAB.6 + [.]         2       252       788.5460       2352.376.6.09       2351.376.0       -0.6955       0       9       R.QQSLAVIDETIMECEDVARAB.6 + [.]       -0.6927       0       R.QQSLAVIDETIMECEDVARAB.6 + [.]       -0.6927       R.QSLAVIDETIMECEDVARAB.6 + [.]       -0.69276       252       0.000       -0.69276       0       1       R.QGSLAVIDETIMECEDVARAB.6 + [.]       -0.69276       252       0.000       R.QCSLAVIDETIMECEDVARAB.6 + [.]       R.QSLAVIDETIMECEDVARAB.6 + [.]       R.RQSLAVIDETIMECEDVARAB.6 + [.]       R.RQSLAVIDE											100	
2       252       290-2187       2287.0341       2264.2295       -0.4954       0       91       7.4-988       1       0       R.OGSANTDOETIMACTOWARA.6         2       252       920-2187       2724.2205       2261.2205       221       1       R.OGSANTDOETIMACTOWARA.6       1       R.OGSANTDOETIMACTOWARA.6         2       222       920-9100       2741.7205							-		4.9			
2       226       208.7208       224.6460       245.510       -0.5104       0       66)       1       U       R_QOSAVIDETINECTOMENTATION (= [127.0212] at N=term 0]         2       24       93.0100       271.2200       0.055.00       (41)       1       N_COSAVIDETINECTOMENTATION (= [127.0212] at N=term 0]         2       225       200.5878       2758.7415       2759.5582       -0.6157       90       1       N_COSCAVID(123080010VII(GGGR.K + Accetyl (N=term)) 0xidation (N)) [_129.5258       at [2]         2       211       1078.6127       2222.8750       223.5678       2552.967.61       223.5678       -0.6167       90       1       R_COSCAVID(123080010VII(GGGR.K + Accetyl (N=term)) 0xidation (N)) [_129.5258       at [2]         2       211       1078.6127       223.2876       233.562       -0.6167       0       16       1       R_COSCAVID(13080010VII(GGGR.K + Accetyl (N=term)) 0xidation (N)) [_129.5258       at [2]         2       211       1078.6127       232.876       233.5627       -0.6167       0       16       1       R_COSCAVID(13080010VII(GGGR.K + Accetyl (N=term)) 0xidation (N)) [_129.5258       at [2]       16       1       R_COSCAVID(13080010VII(GGR.K + Accetyl (N=term)) 0xidation (N)) [_129.5258       at [2]       16       1       R_COSCAVID(13080010VII(GGR.K + Accetyl (N=term)) 0												
02       224       914.0140       2741.7240.72400.7440.74.1400.7440       2741.7440.7400.7400.74.1400.74									7.46.08		-	
213       928.5878       2759.7455       2759.7582       -0.6667       9       9       1       R_QGCCQDATQCISMEDDUTLGGGE.K + Acetyl (N-term)) Oxidation (N); [.0.9526 at E2]         211       1078.6327       3232.8763       3233.5659       -0.6667       0       16       1       R_AQGUCQDATQCISMEDEDUTLGGGE.K + Acetyl (N-term)) Oxidation (N); [.0.9526 at E2]         221       1078.6327       3232.8763       3233.5659       -0.6667       0       16       1       R_AQGUCQDATQCISMEDEDUTLGGGE.K + Acetyl (N-term)) Oxidation (N); [.0.9526 at E2]         221       1050.6327       3232.8763       3233.5659       -0.6667       0       16       1       R_AQGUTATQCISMEDEDUTLGGGE.K + Acetyl (N-term)) Oxidation (N); [.0.9526 at E2]         221       1050.6327       3232.8763       3233.5659       -0.6667       0       16       1       R_AQGUTATQCISMEDEDUTLGGE.K + Acetyl (N-term)) Oxidation (N); [.0.9526 at E2]         23       450.607       70.1567       10       10       16       1       R_AQGUTATQCISMEDUTLGGE.K + Acetyl (N-term))       16       1       16       1         24       450.607       70.1567       30       3       17       1       1.677741256.7       1       1       16       1       1       1       1       1       1       1							1			1		
211       10776.6327       3233.5629       -0.6667       0       16       1       R.AcQUISEEDTLSLVTADHSMMTSFGOVFLE.6         2201       MSUSS       7462       Score: 362       Matches: 27(8)       Sequences: 15(8)         4lbaline phosphataxe, placental-like OS-Homo: saplane: GU-ALPPL2 PE-1 SV-4       Check to include this hit in error tolerant search         Query Observed Pr(cept)       Pr(calc)       Delta Riss Score: Expect Rank Unique Peptide         22       402.6007       923.5186       -0.1404       0       31       0.17       1       R.FVFMLSE.T         31       517.1700       1023.5075       1023.5084       -0.227       0       70       4e-05       2       R.0537FGLAUGK.A         31       545.617       1089.518       1089.5287       -0.227       0       70       4e-05       2       R.0537FGLAUGK.A												
Alkaline phosphetase, placental-like Od-Homon Saplans GM-ALPPL2 PE-1 SV-4         Check to include this hit in error tolerant search         Query Observed Mr(capt) Mr(calc) Delta Miss Score Expect Bank Unique Peptide         12       402.6807 922.1040 933.5116 -0-1660 0 33 0.17 1 R.FPVEALSK.T         13       517.1706 1023.0375 1022.5064 -0-2227 0 0 70 4e-05 2 R.GSIFF0LAPGK.A         13       545.6817 1020.4818 1089.5815 -0.227 0 (53) 1 R.GSIFF0LAPGK.A									16	1		
22         445.6487         923.5446         923.5116         -0.1669         9         3         0.17         1         R.FWYMARG.T           31         517.1766         102.5757         102.5647         642.95         2         R.0551704406.1A           32         545.6431         1089.5491         1089.5451         -0.2327         0         (53)         1         R.0551704406.1A	888	274 275 281 ecen_m Alkali	920.5878 1078.6327 (2537) Mas ne phosphat	2758.7415 3232.8763 is: 57626 iase, placer	2759.3582 3233.5629 Score: 362 stal-like 05	-0.6167 -0.6867 Match +Hono sap	iens	(41) 90 10	Sequence	15	(8)	R.QEGCQDIATQLISNEDIDVILGGGR.K + Oxidation (M); [ <u>-79.9568</u> at C4] R_QEGCQDIATQLISNEDIDVILGGGR.K + Acetyl (N-term); Oxidation (M); [ <u>-0.9476</u> at E2]
41         517,1700         1052,3375         1052,5604         -0.2229         0         70         4e-05         2         R.0551F0LAPOK.A           51         545.6819         1089,5819         -0.2327         0         (53)         1         R.0551F0LAPOK.A	,						Miss			Rank	Unique	
52 545.6419 1089.3491 1089.5819 -0.2327 0 (51) 1 R.055190LAPGK.A + [+57.0215 at 52]							0			1		
									48-05	2		
por pt ved(0)			345,6819	1089,3491	1089.5819	+0.2327		(53)				#:0251F0LAPOK:A + [ <u>*27:0212</u> at 52]
	ncipt	+0+0(0)					1					

As is methylation ay T8 for query 211

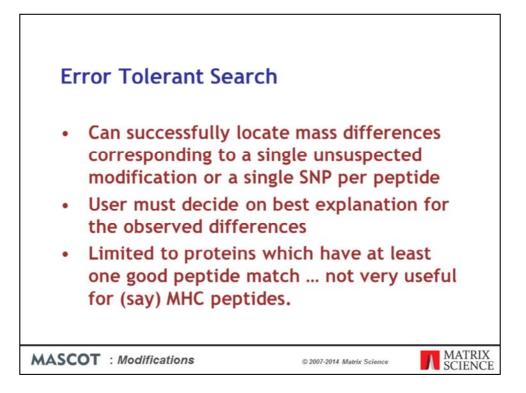
. 7	C	🗋 www.m	atrixscience	.com/cgi/m	aster_resi	alts.p	l?file=	_%2Fdata	1%2F	201409	915%2FFTgcfieOLdat 😥 🛃 🗉
	65	567.6567	1133.2987	1133.5499	-0.2511	0	44	0.011	1		R.GNEVISV9NR.A + Oxidation (H)
8		614.2001	1226.3856	1226.6329	-0.2473		27	0.56	1	U	K.LGPEIPLAMOR.F = Oxidation (M)
8	100	653.2101	1304,4057	1304.6837	-0.2780	0	(#7)	5.8e-07	1		K. GNFQTIGLSAAAR. F
2	124	710.2235	1418,4324	1418.7266	-0.2942		95				K_GNFQTIGLSAAAR.F - [-114.0429 at N-term G]
×.	126	726.1806	1450,3465	1450.6477	-0.3011	0	73	1.2e-05	1		R. NRYSDADVPASAR.Q
2	122	499.1349	1494.3828	1494.6694	-0.2866	0	88		1		L.DPSLEEMTEAALR.L + 2 Oxidation (R)
1	126		1507.3582		-0.3109	0	(44)		1		R_MMYSDADVPASAR.Q + [+57,0215 at N-term N]
8	145	526.1538	1575.4396	1575,7814	-0.3418	٠	(61)		1		R.ALTETIMEDDAIER.A + [_48.0000 at F8]
8	156		1639,4420	1639.7763	-0.3343	0	106	6.2e-09	1		R.ALTETIMFODALER.A . Oxidation Law
8	165		1680,4474	1680.8029	-0.3554	0	(75)		1		R_ALTETINFDOATER.A + Oxidation Possible Assignments: [# A]
×	170		1726.5629		-0.3664	0	44	0.0092	1		K.AYTVLLYGAGPGYVLK.D
8	175	586,4951	1756,4635		-0,3766		(48)		1		G.IIPVEEENPDFWNR.E Phe->Val (F) [-48.0000]
æ	176		1756.4705		-0.3715	0	#3		1		G. TIPVELENPOPUNR. E
8	179		1777.4285		-0.3478	•	45		1		K.HVPDSGATATAYLCGVg.G = [-31.9352 at C-term K]
8	294		1910.4729	1910.8601	-0.3872	•	30	0.23	1	0	R.OSTLDPSLMEMTEAALR.L + 2 Oxidation (M)
8	201		1949,6055		-0.4190	•	85	6.5e-07	1		K.NLIIFLGDGGOVSTVTAAR.I = Oxidation (M)
8	2.9.2		1950.4534		-0.4021	0	(27)	0.41	1		K. DGARPONTESESGSPEVR. Q
8	211	696,1792			0.6327	•	(72)				K.DGARPDVIESESGSPEYR.Q = [ <u>=14.0157</u> at T#]
8	213	664.5518	1990.6336	1991.0510	-0.4174		(58)		1		K_NLIIFLGDGHGVSTVTAAR.I + Oxidation (M); [+41.0266 at N-term N]
8	215	1001.2027	2000.3908	2000.8058	-0.4150			4.1e-05	1		R.BETPOPEYPOOTSQGETR.L = Oxidation (M)
8	217	667.8046	2000.3919	2000.8058	-0.4139	•	76	4.9e-06	-	. U	<pre>#.HGTPDPEYPDDYSQGGTR.L + Oxidation (#)</pre>
8	218	670.1561	2007,4466	2007.8770	0.4304		75		1		<pre>k_QGARPDVTESESGSPEYR.Q + Acetyl (N-term); [+15.0109 at N-term 0]</pre>
8	222	681.8205	2042,4397	2041.8324	0.6073	0	(61)			U	R_HGTPDPEvPDOvSQGGTR.1 + Acetyl (N-term); Oxidation (H); [-0.2840 at E7]
8.8	224	1029,7081	2057,4016	2057.8273 2132.1340	-0.4256		(45)	4.9			R_HETPOPEYPOOYSQUEER.L + Oxidation (M); (+57,0215 at N-term M)
ŝ	227	784,5440	2350,6103	2351.1030			(69)	4.9			K.LGPEIPLAMORFPYVALSK.T + Oxidation (M)
8	253		2367,6341		-0.4927		94	7.40-08			R_QQSAVFLDEETHAGEDVAVFAR.G + [-17.0265 at N-term Q]
2	260		2424.6406	2425,1510	0.5104		(66)	1.46.08			R.QQSAVFLDEETHAGEDVAVFAR.G R_QQSAVFLDEETHAGEDVAVFAR.G + [+57,0215 at N-term 0]
ŝ	274	914,9160	2741.7263		0.4956		(41)				R.QEGCQDIATQLISMEDIDVILGGGR.K + Oxidation (M); [+79,9568 at C4]
ĩ	275		2758.7415		-0.6167		90				R_QEGCQDIATQLISMEDIDVILGGGR.K + Acetyl (N-term); Oxidation (M); [_0,9476 at E2]
8	261		3232.8763		-0.6867	0	10	16	1		R.AGQLISEEDILSLVTADHSHVFSFGGYPLR.G
		ne phosphat		Score: 362 stal-like 05 error tole	+Hono sap	iens		Sequences PPL2 PE+1		8)	
3	Query 22	Observed 462.6807	Mr(expt) 923.3468	Mr(calc) 923.5116	Delta -0.1649	Miss	Score	Expect 0	Rank	Inique	Peptide R.#PYVALSK.T
	41	517.1760	1032.3375		-0.2229	0	70	44-05	2		R. 0551F0LAPOK, A
	53				+0.2327	0	(53)		1		R.0551F0LAPOK.A + [+57.0215 at 52]
noiet	V040(0)						1.0	144			C 100 C 100 C

In other cases, the match may be good, but the assignment is not believable. Query 145 is listed with a substitution at F8 causing a loss of 48 Da. This seems unlikely because we have 2 other matches to the same peptide without any substitution. What else could it be? Well, notice that the other two matches are both oxidised at M7. If we suppose this peptide is also oxidised, then the mass shift becomes -64, which is a well-known loss for oxidised methionine, (loss of methanesulfenic acid). This would seem a much more likely explanation for this match.

It is important to understand that the error tolerant search finds new matches by introducing mass shifts at different positions in the database sequences. The match may be very strong, but figuring out a credible assignment can require a bit of detective work.



You should also look at the other matches to the same query when trying to decide whether to accept a match or not. In this search, Acetyl (N-term was a variable modification. The error tolerant search got the highest score for this spectrum by including this modification and at the same time subtracting 42 Da at E5. Much more believable to take the original match from the first pass search, which is a match to the unmodified peptide with a slightly lower score



In summary, an error tolerant search

•Can successfully locate mass differences corresponding to a single unsuspected modification or a single SNP per peptide

•User must decide on best explanation for the observed differences

•Limited to proteins which have at least one good peptide match ... not very useful for (say) MHC peptides