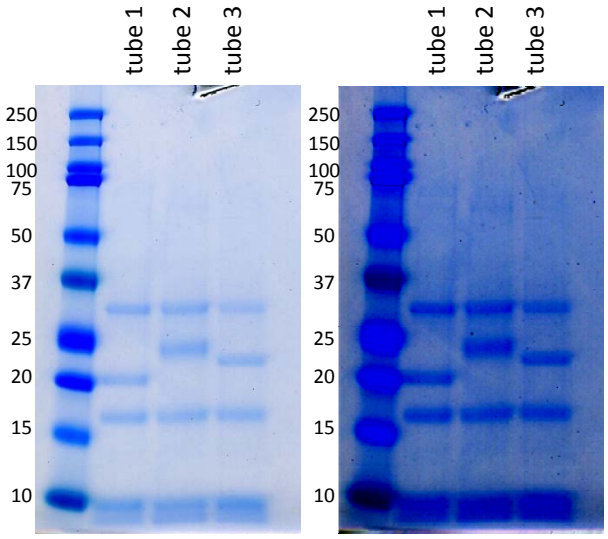
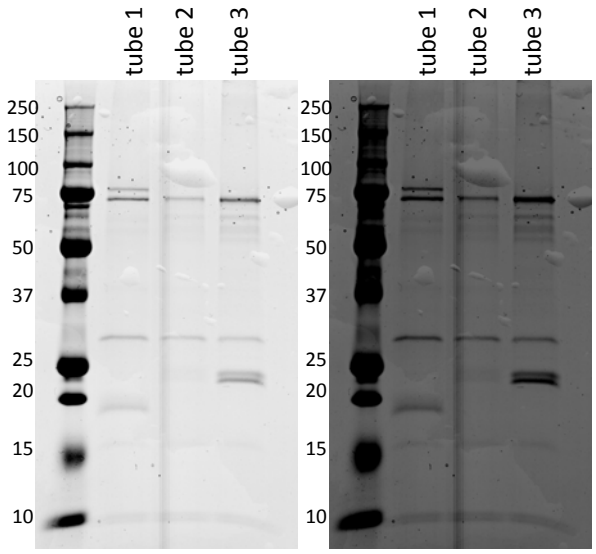
Examples from the samples processed in two separate laboratories



Each tube was resuspended in 20uL 20mM ammonium bicarbonate. 10uL was mixed with 3.3 uL 4x SDS sample buffer (Invitrogen), heated for 10 min and resolved on a 10% continuous SDS-PAGE gel (NuPAGE, Invitrogen) using the MES buffer system, followed by staining with colloidal comassie.

over-exposure



Each tube was resuspended in 12uL TNE buffer (50mM Tris-HCl, pH 7.6, 150mM NaCl, 2mM EDTA, pH 8.0, and 1% (v/v) NP-40). 5uL of each was mixed with 5uL 2x SDS sample buffer (0.125M Tris-Cl, pH 6.8, 4% SDS, 20% v/v Glycerol, 0.2M DTT, 0.02% Bromophenol Blue) and heated for 5mins. Samples were resolved on a 10-20% gradient SDS-PAGE tris-glycine gel and stained with colloidal coomassie.

over-exposure

Suggestions for processing ^{15}N-labelled proteins digest data for database searching:

Some search engines use a list of elemental masses to calculate peptide masses. In these cases it may be convenient to (temporarily) edit the mass of N to 15.xx (exact)/15.xx (average), then restore the value after completing the searches

Specific tips:

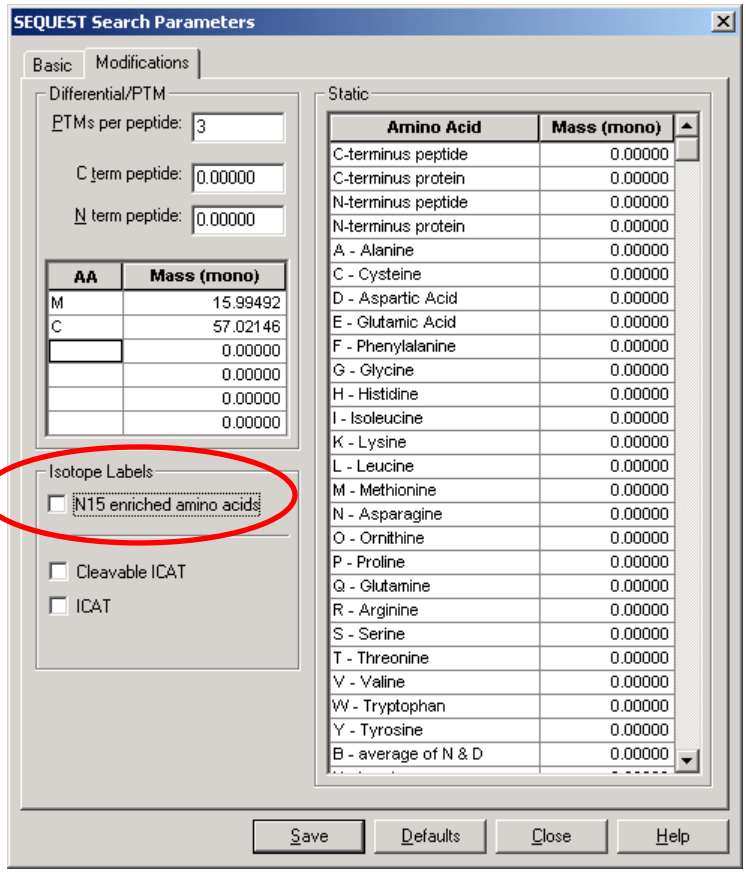
Thermo – Bioworks Browser 3.3.1:

1. Open the file for Bioworks Browser.

2. Under “Actions” select “Sequest Search”.

3. Set up the Search Parameters by clicking on “Edit” to the right of the Search Parameter Selection box.

4. Select the “Modifications” tab. Adjust as desired, and click the N15 checkbox. You may notice several “Mass” values in the Static table adjusting to the selection.



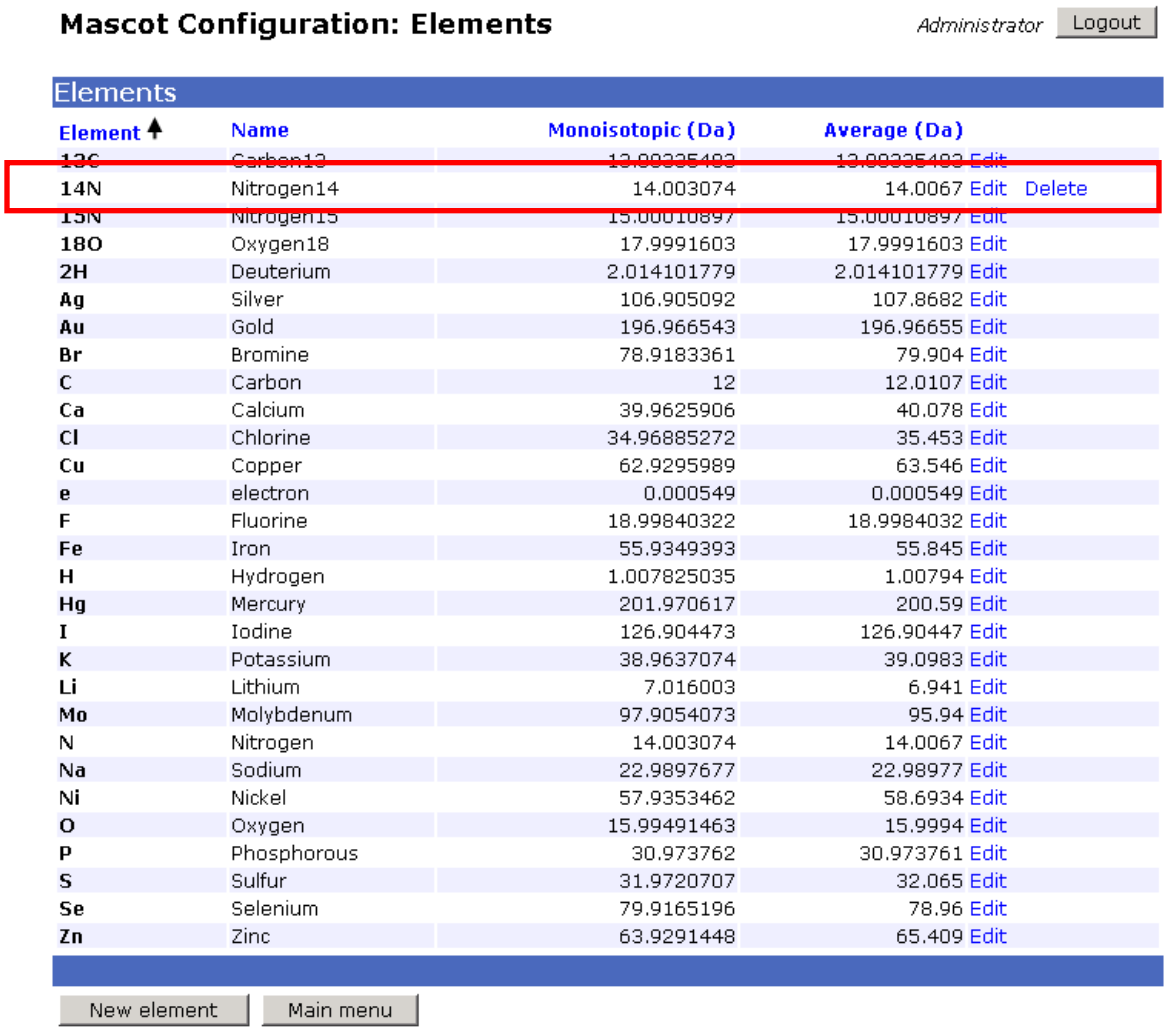
5. Check the general search parameters under the “Basic” tab, save the parameter file, and proceed with the search.

Mascot 2.2

Note: If your Mascot installation has security enabled, you will probably need to log onto it as “admin”.

1. Navigate your internet browser to your Macot server’s home page and select “Configuration Editor”.

2. Select “Element”. The page should like much like this:



Mascot Configuration: Elements

Administrator Logout

Elements

Element	Name	Monoisotopic (Da)	Average (Da)	
~~13C~~	~~Carbon13~~	~~13.00335483~~	~~13.00335483~~ Edit	
14N	Nitrogen14	14.003074	14.0067 Edit	Delete
~~15N~~	~~Nitrogen15~~	~~15.00010897~~	~~15.00010897~~ Edit	
18O	Oxygen18	17.9991603	17.9991603 Edit	
2H	Deuterium	2.014101779	2.014101779 Edit	
Ag	Silver	106.905092	107.8682 Edit	
Au	Gold	196.966543	196.96655 Edit	
Br	Bromine	78.9183361	79.904 Edit	
C	Carbon	12	12.0107 Edit	
Ca	Calcium	39.9625906	40.078 Edit	
Cl	Chlorine	34.96885272	35.453 Edit	
Cu	Copper	62.9295989	63.546 Edit	
e	electron	0.000549	0.000549 Edit	
F	Fluorine	18.99840322	18.9984032 Edit	
Fe	Iron	55.9349393	55.845 Edit	
H	Hydrogen	1.007825035	1.00794 Edit	
Hg	Mercury	201.970617	200.59 Edit	
I	Iodine	126.904473	126.90447 Edit	
K	Potassium	38.9637074	39.0983 Edit	
Li	Lithium	7.016003	6.941 Edit	
Mo	Molybdenum	97.9054073	95.94 Edit	
N	Nitrogen	14.003074	14.0067 Edit	
Na	Sodium	22.9897677	22.98977 Edit	
Ni	Nickel	57.9353462	58.6934 Edit	
O	Oxygen	15.99491463	15.9994 Edit	
P	Phosphorous	30.973762	30.973761 Edit	
S	Sulfur	31.9720707	32.065 Edit	
Se	Selenium	79.9165196	78.96 Edit	
Zn	Zinc	63.9291448	65.409 Edit	

New element Main menu

EXCEPT that the entry above for 14N will be missing. It is convenient to create this entry as a convenient place to store the 14N masses during periods when the N values are set to 15N!!

3. Using the “New Element” button (bottom of page) create the 14N element as shown above. Copy the values from the entry for “N” on the table.

4. After you are sure the 14N entry has been created, cleck “Edit” on the right side of the entry for “N” and enter the values from the 15N line on the table into in “N” entry.

5. Perform the search(es) of files expected to contain 15N-labelled peptides.

6. Be sure to go back and reset the masses for the “N” entry to that of 14N when you are finished with the 15N searches!!

Older versions of Mascot:

You may need to change the mass of N from 14 to 15 in the ‘masses’ file that MASCOT reads (in the C:\Inetpub\MASCOT\config directory), and also change the masses of all of the AA’s according to their N content. The best thing to do would be to create a second masses file for 15N and swap between this and the standard file as required.

An effective way to do this would be to first create two master stock files, one labeled ‘masses 14N’ and the other ‘masses 15N’. Then:

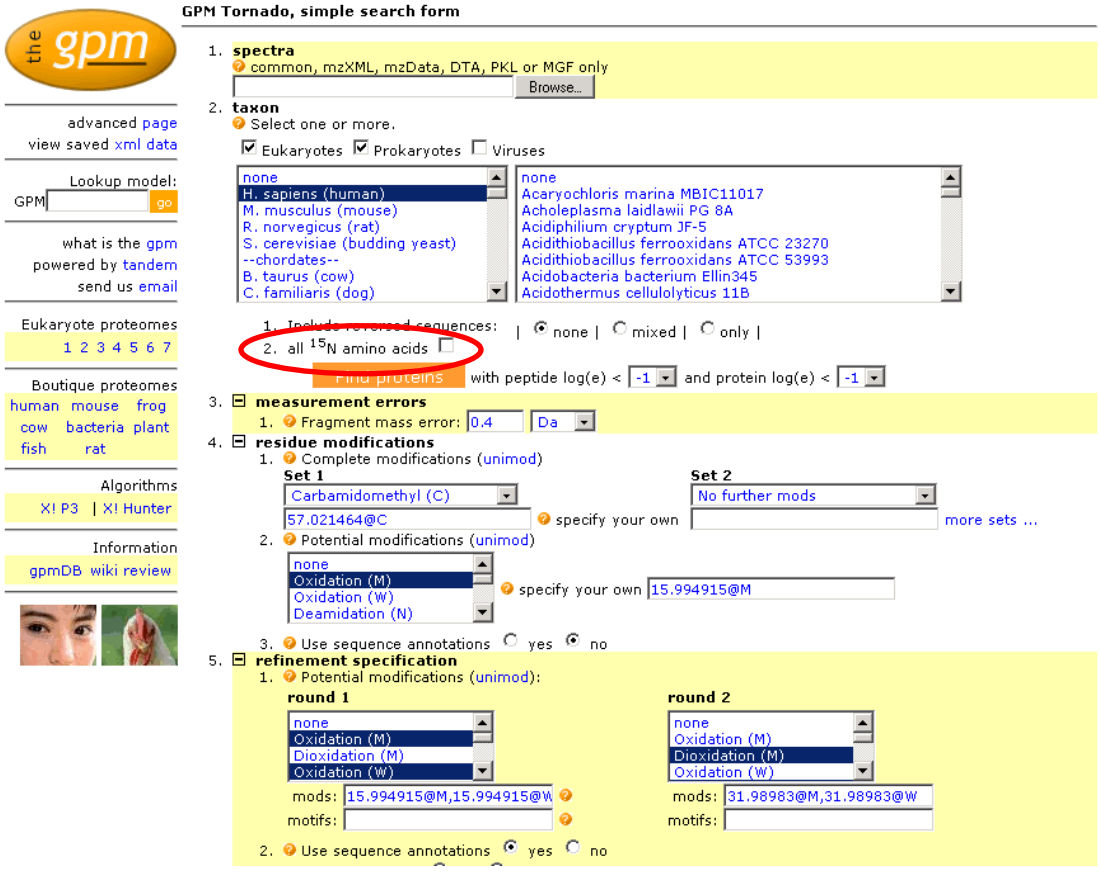
1) make a copy of “masses 15N”
2) delete “masses”
3) change name of “masses 15N” to “masses”
4) stop and restart the Mascot server and perform your 15N searches
5) when finished, repeat with “masses 14N” as above to restore to conventional searches.

The GPM (free search tool on the web)

1. Navigate to www.thegpm.org

2. Go to a search page (selection on the left).

3. Use the “all 15N amino acids” checkbox:



4. Make other selections, be sure to select a datafile in one of the indicated formats, and go!