

Comprehensive support for quantitation

MASCOT

{MATRIX}
{SCIENCE}

One of the major new features in the current release of Mascot is support for quantitation. This is still work in progress. Our goal is to support all of the popular methodologies.

Quantitation - Overview

Protocol	Description	Examples
reporter	Specific reporter ion peaks within a single MS/MS spectrum	iTRAQ, ExacTag, TMT
precursor	Extracted ion chromatograms for related precursors within a single dataset	ICAT, SILAC, ¹⁸ O, ICPL, AQUA, Metabolic
multiplex (Neubert et. al.)	Pairs of sequence ion fragment peaks within a single MS/MS spectrum	SILAC, ¹⁸ O
replicate	Extracted ion chromatograms for identical precursors across two or more datasets	Label-free
empai (Ishihama et. al.)	Protein coverage in a database search result	
average	Extracted ion chromatograms for selected peptides per protein within a single dataset	

MASCOT : Quantitation

© 2007 Matrix Science



To make this task manageable, we have classified the various approaches into a limited number of protocols. So far, we have identified 6 distinct protocols. If anyone can see a method that doesn't fit to one of these, we'd be very grateful for details.

Reporter is quantitation based on the relative intensities of fragment peaks at fixed m/z values within an MS/MS spectrum. For example, iTRAQ or Tandem Mass Tags

Precursor is quantitation based on the relative intensities of extracted ion chromatograms (XICs) for precursors within a single data set. This is by far the most widely used approach, which can be used with any chemistry that creates a precursor mass shift. For example, ¹⁸O, AQUA, ICAT, ICPL, Metabolic, SILAC, etc., etc.

Multiplex is quantitation based on the relative intensities of sequence ion fragment peaks within an MS/MS spectrum. This is a novel approach, which can be used with any chemistry that labels one peptide terminus, creating a small mass shift, such as ¹⁸O or SILAC under certain conditions.

Replicate is label free quantitation based on the relative intensities of extracted ion chromatograms (XICs) for precursors in multiple data sets aligned using mass and elution time.

emPAI is quantitation for the proteins in a mixture based on protein coverage by the peptide matches in a database search result.

Average is quantitation for the proteins in a mixture based on the application of a rule to the intensities of extracted ion chromatograms (XICs) for the peptide matches in a database search result. For example, the average intensity for the three strongest peptide matches per protein

The rows with a blue background are the protocols that are fully implemented in Mascot 2.2

Quantitation - Overview

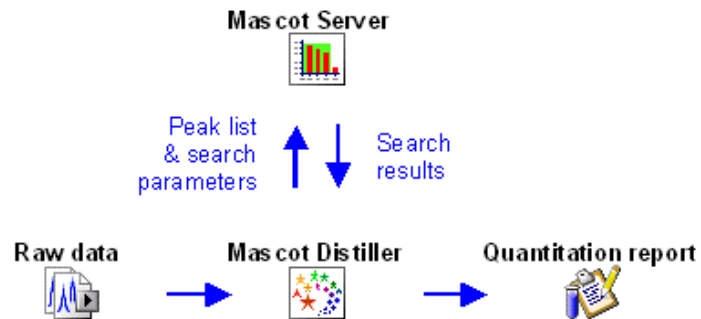
- **Quantitation methods that only require information available in the MS/MS peak list are supported in Mascot Server 2.2**
 - reporter
 - multiplex
 - emPAI
- **Methods that require additional information from the raw data file require Mascot Distiller + Quantitation Toolbox ... not yet released**
 - precursor
 - replicate
 - average

The common factor for these protocols is that all of the information required for quantitation is contained in the peak list.

The other three methods require additional information from the raw data file, either because it is necessary to integrate the elution profile of each peptide or because information is required for multiple peaks in the survey scan. These methods require that the raw data files are processed using Mascot Distiller.

Quantitation - Overview

Workflow for methods that require additional information from the raw data file, (precursor, replicate, average)



MASCOT : Quantitation

© 2007 Matrix Science

MATRIX
SCIENCE

For methods that require additional information from the raw data file, the workflow looks like this. The raw data file is processed in Distiller and the search submitted to Mascot. When the search is complete, the results are returned to Distiller. The quantitation report can then be generated in Mascot Distiller, which has access to both the Mascot search results and the raw data.

The Quantitation Toolbox for Distiller is still under development. We will try to get it released as soon as we can.

Mascot 2.2 Changes

Named quantitation methods

- A method defines how to search and report data from a quantitation experiment

Modifications defined in unimod.xml

- Residues and modifications all defined in terms of elemental composition

Metabolic labelling

- e.g. ^{14}N and ^{15}N

Exclusive modifications

- A choice of fixed modifications

Let me emphasise that the changes to the Mascot search engine are complete and released.

The set of quantitation methods is defined in a new XML configuration file, called `quantitation.xml`. As with other configuration files, this file lives on the Mascot Server and is downloaded by Mascot Distiller and other clients as required.

The introduction of quantitation has required changes in the way that modifications are handled. Mascot now takes its modification definitions direct from an XML representation of the Unimod database.

One factor that forced this change was the need to support metabolic labelling, in which the isotopic label is present throughout the peptide backbone. This requires residues and modifications to be defined and manipulated as elemental compositions.

We have also introduced exclusive modifications, which can be thought of as a choice of fixed modifications. In many quantitation experiments, separate samples are derivatised then pooled. Thus, a given peptide may carry one or the other set of modifications, but never a mixture of both. Some people use the term "binary" for this type of specificity. We prefer exclusive because binary implies only two possibilities. The real importance of this is that it keeps the search space small, and avoids the 'combinatorial explosion' that can happen with too many variable modifications

Quantitation

Simplicity
for user

The screenshot shows the Mascot MS/MS Ions Search web interface. The browser window title is "Matrix Science - Mascot - MS/MS Ions Search - Microsoft Internet Explorer". The address bar shows "http://www.matrixscience.com/cgi/search_form.pl?FORMER=2&SEARCH=MIS". The page header includes "HOME | WHAT'S NEW | MASCOT | HELP | PRODUCTS | SUPPORT | TRAINING | CONTACT" and a search box. The main heading is "MASCOT MS/MS Ions Search". The form includes fields for "Your name", "Email", and "Search title". The "Database" is set to "SwissProt". "Taxonomy" is set to "All entries". "Enzyme" is set to "Trypsin". "Allow up to" is set to "1 missed cleavages". The "Fixed modifications" list includes Acetyl (K), Acetyl (N-term), Acetyl (Protein N-term), Amidated (C-term), and Amidated (Protein C-term). The "Variable modifications" list includes Acetyl (K), Acetyl (N-term), Acetyl (Protein N-term), Amidated (C-term), and Amidated (Protein C-term). The "Quantitation" dropdown is set to "ETRAO 4plex". "Peptide tol. ±" is set to "1.2 Da" and "# ±C" is set to "0". "MS/MS tol. ±" is set to "0.6 Da". "Peptide charge" is set to "1+". "Data file" has a "Browse..." button. "Data format" is set to "Mascot generic". "Instrument" is set to "Default". "Decoy" is unchecked. "Precursor" is set to "m/z". "Error tolerant" is unchecked. "Report top" is set to "AUTO hits". There are "Start Search ..." and "Reset Form" buttons. The footer contains "Copyright © 2006 Matrix Science Ltd. All Rights Reserved." and "Internet".

MASCOT : Quantitation

© 2007 Matrix Science



We wanted to keep the user interface simple. Quantitation adds a huge number of choices and parameters, but there is no point in exposing all of these in the search form.

The approach we have chosen is encapsulate these choices and parameters into named quantitation methods. This means that the search form has just a single new control, which replaces the old ICAT checkbox.

Methods that have [MD] at the end are the ones that require Mascot Distiller

Quantitation

The quantitation methods are defined in a single XML configuration file

- quantitation.xml
- Browser based editor
- Add new methods as required
- Used by Mascot Server and Mascot Distiller

The configuration file that encapsulates the choices and parameters for each quantitation method is called quantitation.xml. This is an XML file, and there is a browser based editor for modifying methods and creating new ones. quantitation.xml lives on the Mascot server and is read by both the search engine and Mascot Distiller

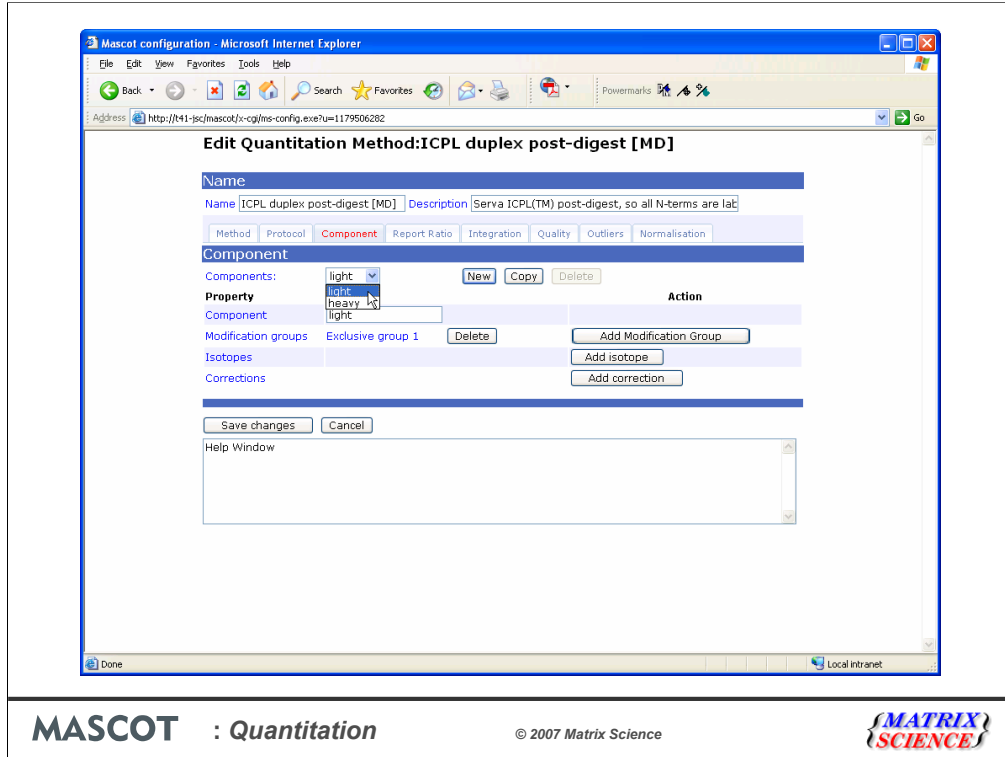
Mascot Configuration: Quantitation Methods

Quantitation Methods

Name	Protocol	Copy	Delete	Print
None	null			
ITRAQ 4plex	reporter	Copy	Delete	Print
ITRAQ 8plex	reporter	Copy	Delete	Print
18O labeled multiplex	multiplex	Copy	Delete	Print
SILAC K+6 R+6 multiplex	multiplex	Copy	Delete	Print
TMT 6plex	reporter	Copy	Delete	Print
ICAT ABI Cleavable [MD]	precursor	Copy	Delete	Print
ICPL duplex pre-digest [MD]	precursor	Copy	Delete	Print
ICPL duplex post-digest [MD]	precursor	Copy	Delete	Print
SILAC K+6 R+10 [MD]	precursor	Copy	Delete	Print
18O corrected [MD]	precursor	Copy	Delete	Print
15N Metabolic [MD]	precursor	Copy	Delete	Print

Applied Biosystems ITRAQ(TM) 8-plex reagent

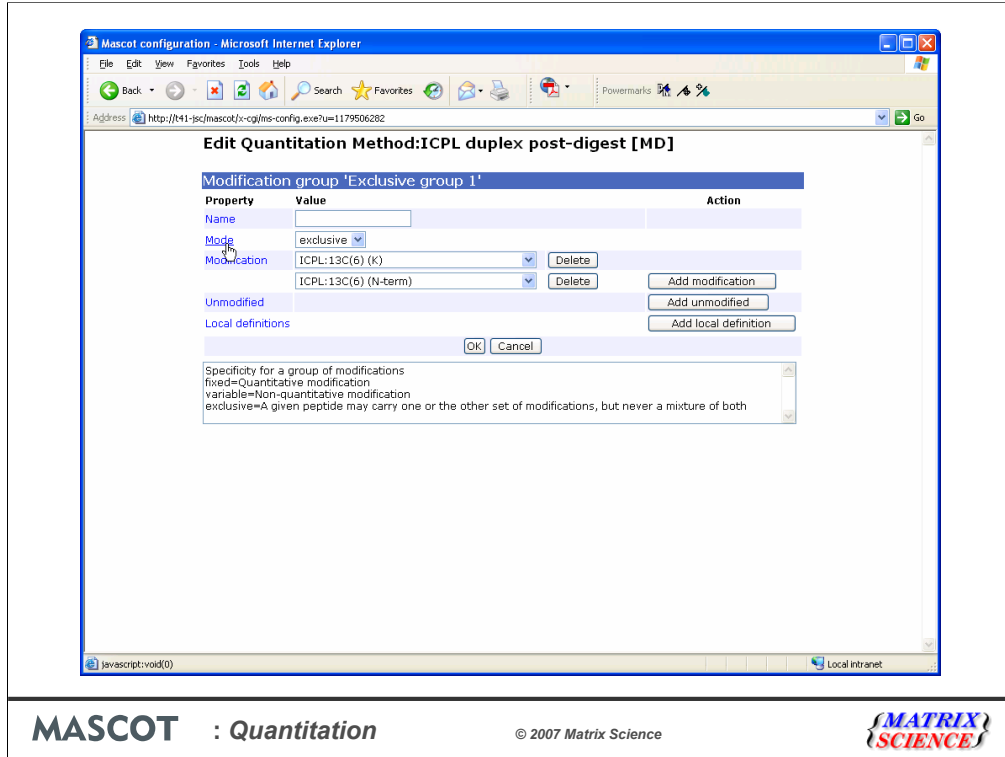
The new, browser-based Configuration Editor, provides an interface to all the Mascot configuration files. In the case of quantitation, you can edit an existing method or make a copy of it as the basis for a new method



For each method, a tabbed dialog is used to navigate between property pages. In many cases, the property pages correspond to XML elements, but sometimes elements have been combined onto a single page or split across multiple pages so as to give a balanced layout.

Here, we can see a duplex ICPL method. The unlabelled and labelled components have been called heavy and light, but you are free to choose your own names so as to make the final report as clear as possible.

If we choose the heavy component, then click on the Modification group link



We see that the heavy component corresponds to these two modifications. In this particular method, the sample has been labelled after digestion, so the amino terminus modification is found on every peptide. As you can see from the buttons on the right, a component can be defined in terms of modified or unmodified residues or termini. A local definition is a special modification defined within the quantitation method.

The screenshot shows a Microsoft Internet Explorer browser window displaying the Matrix Science website. The address bar shows the URL: http://www.matrixscience.com/help/quant_statistics_help.html. The page title is "Matrix Science - Help - Quantitation: Statistical procedures - Microsoft Internet Explorer". The navigation menu includes: HOME | WHAT'S NEW | MASCOT | HELP | PRODUCTS | SUPPORT | TRAINING | CONTACT. The page content is titled "Quantitation: Statistical procedures".

On this page

- Testing for normality
- Outlier removal
- Protein ratio calculation
- Significant changes
- Quantitation topics
 - Overview
 - Report format
 - Configuration
 - Statistical procedures
 - Reporter protocol
 - Precursor protocol
 - Multiplex protocol
 - Replicate protocol
 - ambPFI protocol
 - Average protocol

Usually, identification and quantitation are performed at the peptide level. The Mascot result report assigns the peptide matches to protein hits, and the ratios for individual peptide matches are combined to determine ratios for the protein hits. The methods provided for calculating a protein ratio from a set of peptide ratios are median, average, or weighted average. The standard deviation of the peptide ratios provides a measure of the uncertainty in the protein ratio.

Since we are dealing with ratios, the average is the geometric mean and the standard deviation is the geometric standard deviation, which is a factor. In other words, the confidence interval is obtained by dividing and multiplying the average by the standard deviation, which is never less than 1.0. For example, if the average is 0.91 and SD(geo) is 1.06 then the confidence interval is 0.86 to 0.96.

Ratios for peptide matches are only reported if various quality criteria are fulfilled, the most important being:

- Peptide modification state
- Minimum precursor charge, (default 1)
- Strength of the peptide match, defined in terms of either a minimum score, a maximum expect value, or the score being at or above either the identity threshold or the homology threshold, (default maximum expect of 0.05)
- Method specific criteria, such as a minimum number of fragment ion pairs for multiplex

A ratio for a protein hit is only reported if the minimum number of peptide matches, is achieved, (default 2). A standard deviation is only reported if the ratios for the peptide matches are consistent with a sample from a normal distribution.

Testing for normality

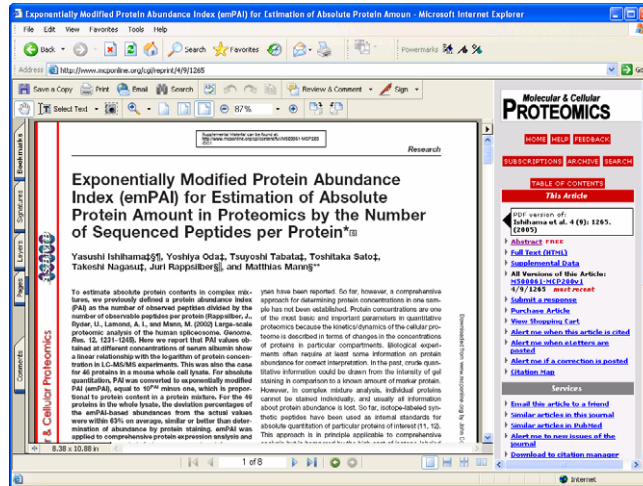
Testing for outliers and reporting a standard deviation for the protein ratio can only be performed if the peptide ratios are consistent with a sample from a normal distribution, (in log space). If the peptide ratios do not appear to be from a normal distribution, this may indicate that the values are meaningless, and something went systematically wrong with the the analysis. On the other hand, it may indicate something interesting, like the peptides have been mis-assigned and actually come from two proteins with very different ratios, so that the distribution is bimodal.

Shapiro-Wilk W test

At the bottom of the page, there is a footer with the text: "MASCOT : Quantitation © 2007 Matrix Science" and the Matrix Science logo.

We have taken trouble to ensure that appropriate statistical procedures are correctly used. For example, we test that a set of peptide ratios is consistent with a normal distribution before rejecting outliers or reporting a standard deviation. Standard deviations are always geometric, because we are dealing with ratios that conform to a normal distribution in log space.

Quantitation - emPAI



MASCOT : Quantitation

© 2007 Matrix Science



emPAI quantitation offers approximate, label-free, relative quantitation of the proteins in a mixture based on protein coverage by the peptide matches in a database search result. This approach was developed by Ishihama and colleagues

Quantitation - emPAI

- Very simple

$$emPAI = 10^{\frac{N_{observed}}{N_{observable}}} - 1$$

- Very approximate

 - Many assumptions in $N_{observed}$ and $N_{observable}$

- 'Always on'

1. [PPB1_HUMAN](#) Mass: 58259 Score: 452 Queries matched: 17 emPAI: 1.04
Alkaline phosphatase, placental type precursor (EC 3.1.3.1) (PLAP-1) (Regan isozyme) - Homo sapiens
 Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta Miss	Score	Expect	Rank	Peptide
<input checked="" type="checkbox"/> 27	462.6807	923.3468	923.5116	-0.1649	0	33	0.25	1 R.FPYVALSK.T
<input checked="" type="checkbox"/> 41	517.1760	1032.3375	1032.5604	-0.2229	0	71	6.4e-05	1 R.GSSIFGLAPGK.A
<input checked="" type="checkbox"/> 62	564.6804	1127.3463	1127.5764	-0.2301	0	9	1.2e+02	1 R.GFFLVEGGK.I

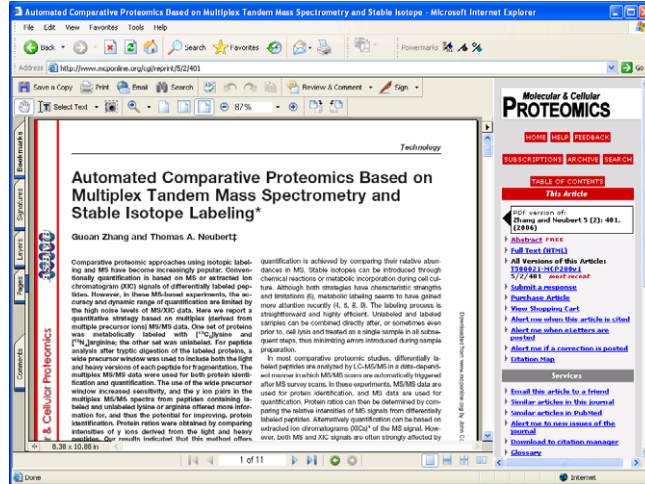
MASCOT : Quantitation

© 2007 Matrix Science

MATRIX
SCIENCE

It is very simple. It is also very approximate, because there are many arbitrary assumptions in the way that the number of observed and observable peptides are calculated. Nevertheless, Ishihama's paper shows that it can be a useful guide to relative amounts. emPAI doesn't require a label or special data processing, so it is always reported in a standard Mascot results report, as long as the number of MS/MS spectra is at least 100

Quantitation - multiplex



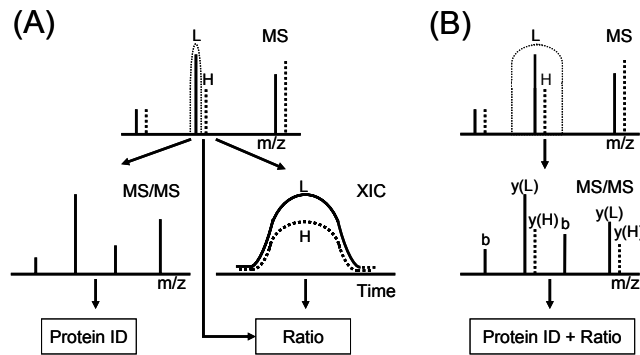
MASCOT : Quantitation

© 2007 Matrix Science



Multiplex is quantitation based on the relative intensities of sequence ion fragment peaks within an MS/MS spectrum. This approach, developed Zhang and Neubert, can be used with any chemistry that labels one peptide terminus and has a reasonably small mass shift.

Quantitation - multiplex



MASCOT : Quantitation

© 2007 Matrix Science

MATRIX
SCIENCE

This diagram, copied from the MCP paper, illustrates how it works. On the left, we have conventional quantitation; the ‘precursor protocol’ in Mascot terms. This requires the precursor intensity for each component to be integrated across its elution profile. In the case of the multiplex protocol, the MS1 transmission window is set wide enough to allow both components through simultaneously, giving a mixed MS/MS spectrum. The relative amounts can be measured from the sequence ions that include the labelled terminus. If the label is on the carboxy terminus, we see the ratios in the y ions.

Quantitation - multiplex

Requirements:

- Label confined to one peptide terminus
e.g. ^{18}O , or SILAC at K or R with trypsin
- MS1 transmission window must be ~ flat over the label delta
- Heavy and light pair must be 'isolated' in survey scan
- Heavy and light must ~ co-elute
- Label must not affect fragmentation kinetics
- Tough to extend to more than 2 components.

The multiplex method has the potential to give excellent precision, because each ratio is represented by multiple sequence ion pairs. On the other hand, the ratio will only be accurate if several constraints are met.

Peptide Summary Report (SILAC example: NG108-EphB2 from Zhang and Neubert) - Microsoft Internet Explorer

Address: http://www.matrixscience.com/cgi/master_results.pl?file=../data/F981133.dat

8. **HNRPV_HUMAN** Mass: 90423 Score: 130 Queries matched: 4
 Heterogeneous nuclear ribonucleoprotein U (hnRNP U) (Scaffold attachment factor A) (SAF-A) (p120) (

Check to include this hit in error tolerant search

Quantitation: Ratio Weighted N SD(geo)
 Heavy/Light 0.794 2 1.021

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Heavy/Light	Peptide
<input checked="" type="checkbox"/> 32	692.2000	1382.3854	1387.7113	-5.3258	0	37	3.1	1	---	K.YNHLGTNTIDDK_M + 13C6_NL_K (C-ter
<input checked="" type="checkbox"/> 34	694.6700	1387.3254	1387.7113	-0.3858	0	(17)	3.2e+02	1	---	K.YNHLGTNTIDDK_M + 13C6_NL_K (C-ter
<input checked="" type="checkbox"/> 51	824.9751	1647.9357	1652.8577	-4.9220	0	(100)	1.3e-06	1	0.811	R.NFILDQTNVSAQAQR_R + 13C6_NL_R (
<input checked="" type="checkbox"/> 52	827.4146	1652.8147	1652.8577	-0.0431	0	104	5.5e-07	1	0.779	R.NFILDQTNVSAQAQR_R + 13C6_NL_R (

9. **EPHB2_HUMAN** Mass: 117417 Score: 130 Queries matched: 4
 Ephrin type-B receptor 2 precursor (EC 2.7.10.1) (Tyrosine-protein kinase receptor EPH-3) (DRT) (Re

Check to include this hit in error tolerant search

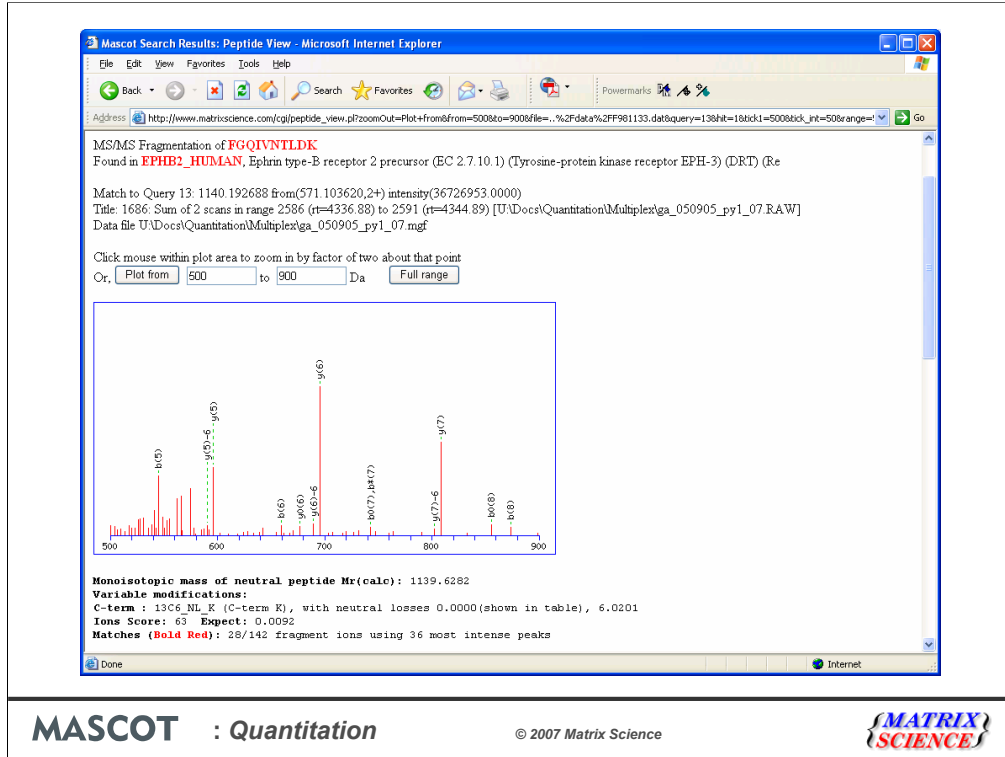
Quantitation: Ratio Weighted N SD(geo)
 Heavy/Light 10.512 2 1.054

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Heavy/Light	Peptide
<input checked="" type="checkbox"/> 13	571.1036	1140.1927	1139.6282	0.5645	0	63	0.0092	1	10.341	K.FGQIVNVLDDK_M + 13C6_NL_K (C-ter
<input checked="" type="checkbox"/> 21	620.9648	1239.9150	1239.6343	0.2807	0	26	45	1	---	R.WTAPAEIQYR_K + 13C6_NL_R (C-ter
<input checked="" type="checkbox"/> 53	837.9240	1673.8335	1672.9277	0.9058	0	43	0.69	1	11.304	K.AMAPLSSGINLPLLR_T + 13C6_NL_R
<input checked="" type="checkbox"/> 88	1124.9100	2247.8054	2247.1042	0.7012	0	17	1.8e+02	1	---	R.TIPDYTSFNTVDENLEAIK_M + 13C6_NL

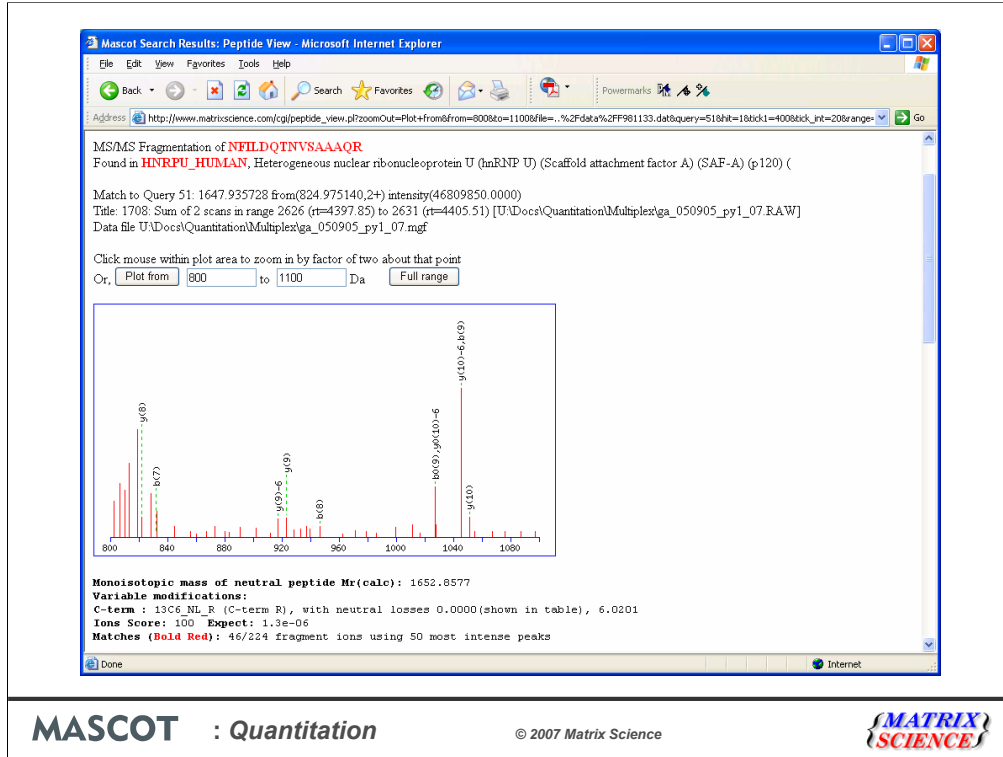
Proteins matching the same set of peptides:
EPHB2_MOUSE Mass: 110688 Score: 130 Queries matched: 4
 Ephrin type-B receptor 2 precursor (EC 2.7.10.1) (Tyrosine-protein kinase receptor EPH-3) (Neural k

MASCOT : Quantitation © 2007 Matrix Science

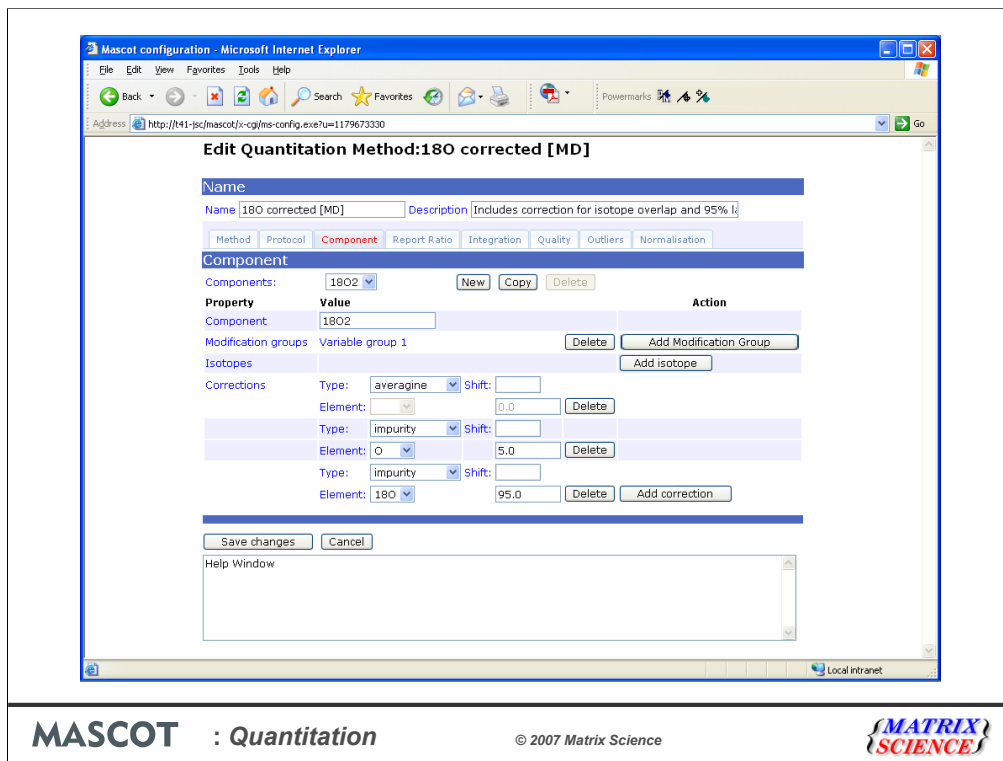
This is an example using a dataset courtesy of Zhang and Neubert. The instrument was an ion trap and the label is 13C(6) SILAC on K and R. If we look at one of the spectra from the Ephrin peptides



We can see that the heavy component has been strongly up-regulated

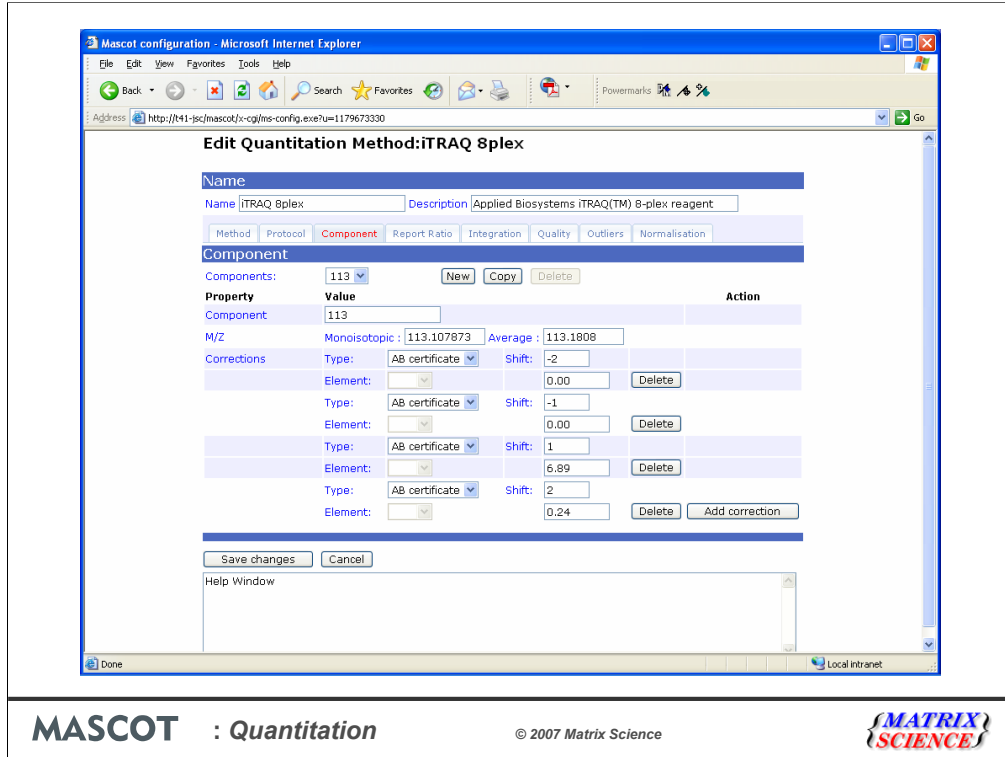


In contrast, this is a spectrum from ribonucleoprotein, which is close to 1:1. This spectrum illustrates the importance of selecting sequence ions that are not overlapped by interfering peaks. In this case, the y(10) pair has to be discarded because the light component coincides with the b(9) ion.



MASCOT : Quantitation © 2007 Matrix Science **MATRIX SCIENCE**

One of the complications of any type of isotope labelling is isotope impurity. It is rarely possible to get 100% enrichment. In the Mascot quantitation schema, this is described by a correction element. An ‘impurity’ correction works "downwards". That is, in this 18O method, some of the intensity of peptides labelled with the 18O label will appear at lower mass values because the heavy water is only 95% enriched. A second type of isotope correction, ‘averagine’, works “upwards”. This describes how some of the intensity will be found at higher mass values because of the natural abundances of heavy isotopes. An averagine correction only matters when the mass delta is small, as in the case of 18O labelling.



A third type of isotope correction is used in iTRAQ, where the correction factors are obtained experimentally, by analysing the isolated reagents. This combines both upward and downward corrections for labels which have complex, multi-isotope compositions

Quantitation - Reporter

SPOT_LABEL	SPOT_NAME	SPOT_TYPE	PRECURSOR MASS	OPMODE
1		Unknown	2336.154	MS-MS 2K
1		Unknown	2617.197	MS-MS 2K
1		Unknown	1252.544	MS-MS 2K
1		Unknown	2514.093	MS-MS 2K
1		Unknown	1344.682	MS-MS 2K
1		Unknown	2463.284	MS-MS 2K
1		Unknown	1233.563	MS-MS 2K
1		Unknown	2479.202	MS-MS 2K
1		Unknown	2447.287	MS-MS 2K
3		Unknown	2542.095	MS-MS 2K
4		Unknown	2486.308	MS-MS 2K

MASCOT : Quantitation

© 2007 Matrix Science



In data processing terms, the reporter protocol is one of the simplest. However, we did find that the peak list exported from the 4000 series data system or submitted to Mascot from GPS Explorer did not have the correct peak areas for the reporter ions. The numbers are different from those used within GPS Explorer for quantitation. We have had to write our own application to export a suitable peak list from the Oracle database. We've released this utility, called TS2Mascot, as freeware, and you can download it from our web site.

So, for iTRAQ, we could launch TS2Mascot and choose Mascot Search ...

Quantitation - Reporter

The screenshot shows the Mascot MS/MS Ions Search web form. The browser window title is "Matrix Science - Mascot MS/MS Ions Search - Microsoft Internet Explorer". The address bar shows the URL: "http://www.matrixscience.com/cgi/search_form.pl?FORMER=26&SEARCH=MIS". The page header includes the Matrix Science logo and navigation links: HOME | WHAT'S NEW | MASCOT | HELP | PRODUCTS | SUPPORT | TRAINING | CONTACT. The main heading is "MASCOT MS/MS Ions Search".

The form fields are as follows:

- Your name:** John Cottrell
- Email:** jcottrell@matrixscience.com
- Search title:** NICKM/New Project Spot Set J5C; Job Run 12384; MS-MS 2kV Positive
- Database:** NCBInr
- Taxonomy:** Homo sapiens (human)
- Enzyme:** Trypsin/P
- Allow up to:** 2 missed cleavages
- Fixed modifications:** Acetyl (K), Acetyl (N-term), Acetyl (Protein N-term), Amidated (C-term), Amidated (Protein C-term)
- Variable modifications:** Acetyl (K), Acetyl (N-term), Acetyl (Protein N-term), Amidated (C-term), Amidated (Protein C-term)
- Quantitation:** iTRAQ 4plex
- Peptide tol. ±:** 0.3 Da
- MS/MS tol. ±:** 0.3 Da
- Peptide charge:** 1+
- Monoisotopic:** Average
- Data file:** h:\LOCALS~1\Temp\ts23D.tmp
- Data format:** Mascot generic
- Precursor:** m/z
- Instrument:** MALDI-TOF-TOF
- Error tolerant:**
- Decoy:**
- Report top:** AUTO hits

Buttons: Start Search, Reset Form

Copyright © 2006 Matrix Science Ltd. All Rights Reserved.

MASCOT : Quantitation

© 2007 Matrix Science



Which brings up the search form. We choose an appropriate quantitation method. We don't need to specify the iTRAQ modifications or the cysteine alkylation, because these are pre-defined in the quantitation method. Submit the search...

Peptide Summary Report (Time flies like an arrow) - Microsoft Internet Explorer

Address: http://www.matrixscience.com/cgi/master_results.pl?file=../data/F981131.dst

MASCOT Search Results

User : Mel Anogaster
Email : mel@bioc.cam.ac.uk
Search title : Time flies like an arrow
MS data file : U:\docs\quantitation\iTRAQ\cantab\deniseef28QSTARReor.mgf
Database : SwissProt 31.6 (257964 sequences; 93947433 residues)
Quantitation : iTRAQ 4plex [method details](#)
Applied Biosystems iTRAQ(TM) reagent
Timestamp : 19 Feb 2007 at 14:31:55 GMT
Protein hits : 115/114 116/114 117/114

	115/114	116/114	117/114	
0.914	1.438	1.809	APLP_DROME	Apolipoporphins precursor (Retinoid- and fatty acid-binding glycoprotein)
1.761	2.245	3.225	VIT3_DROME	Vitellogenin-3 precursor (Vitellogenin III) (Yolk protein 3) - Drosophila
1.891	3.221	1.717	PDI_DROME	Protein disulfide-isomerase precursor (EC 5.3.4.1) (PDI) - Drosophila
1.020	1.191	0.684	UGCG_DROME	UDP-glucose:glycoprotein glucosyltransferase precursor (EC 2.4.1.1)
0.846	1.656	3.233	EFTL_DROME	Elongation factor 1-alpha (EF-1-alpha) (50 kDa female-specific protein)
1.096	4.004	6.741	TOP2_DROME	DNA topoisomerase 2 (EC 5.99.1.3) (DNA topoisomerase II) - Drosophila
0.969	1.221	2.165	RL4_DROME	60S ribosomal protein L4 (L1) - Drosophila melanogaster (Fruit fly)
1.042	2.008	3.426	VDAC_DROME	Voltage-dependent anion-selective channel (Porin) (DmVDAC) - Drosophila
1.422	1.906	1.335	HSP7C_DROME	Heat shock 70 kDa protein cognate 3 precursor (78 kDa glucose-regulated protein)
1.090	1.839	1.182	YL_DROME	Putative vitellogenin receptor precursor (Protein yolkless) (YL) - Drosophila
1.411	2.166	0.928	SPTCA_DROME	Spectrin alpha chain - Drosophila melanogaster (Fruit fly)
0.357	0.831	1.733	ATPB_DROME	ATP synthase subunit beta, mitochondrial precursor (EC 3.6.3.14) - Drosophila
1.051	2.172	2.501	GMBP_DROME	Guanine nucleotide-binding protein subunit beta-like protein (Receptor)
0.747	1.363	2.370	RS3_DROME	40S ribosomal protein S3 - Drosophila melanogaster (Fruit fly)
1.174	1.160	1.554	VIT1_DROME	Vitellogenin-1 precursor (Vitellogenin I) (Yolk protein 1) - Drosophila
1.577	3.060	5.007	BL13_DROME	60S ribosomal protein L13 (BBC1 protein homolog) - Drosophila melanogaster
1.214	1.097	0.717	CALR_DROME	Calreticulin precursor (CRP55) (Calregulin) (HACBP) - Drosophila melanogaster
0.855	1.919	3.548	PFYK_DROME	Pyruvate kinase (EC 2.7.1.40) (PK) - Drosophila melanogaster (Fruit fly)
0.756	0.752	2.068	PABP_DROME	Polyadenylate-binding protein (Poly(A)-binding protein) (PABP) - Drosophila
1.256	1.958	2.789	RL9_DROME	60S ribosomal protein L9 - Drosophila melanogaster (Fruit fly)
---	---	---	LAMC1_DROME	Laminin subunit gamma-1 precursor (Laminin B2 chain) - Drosophila melanogaster

MASCOT : Quantitation © 2007 Matrix Science

And back comes the report. At the top is a summary of the protein ratios. In this example, the method asks for ratios to 114, but you have total flexibility. You can edit the quantitation method to report two pairs, e.g. 115/114 and 117/116, or something more complex, like ratios to the sum of all four channels. Note that you can't do this if you are using our public web site, because this is a shared resource, so you don't have access to the configuration editor.

Peptide Summary Report (Time flies like an arrow) - Microsoft Internet Explorer

Address: http://www.matrixscience.com/cgi/master_results.pl?file=../data/F981131.dat

2. [VIT3_DRONE](#) Mass: 50436 Score: 515 Queries matched: 19 emPAI: 1.13
 Vitellogenin-3 precursor (Vitellogenin III) (Yolk protein 3) - Drosophila melanogaster (Fruit fly)

Check to include this hit in error tolerant search

Quantitation:	Ratio	Weighted	N	SD(geo)
	113/114	1.761	10	1.144
	116/114	2.245	10	1.560
	117/114	3.225	11	NN

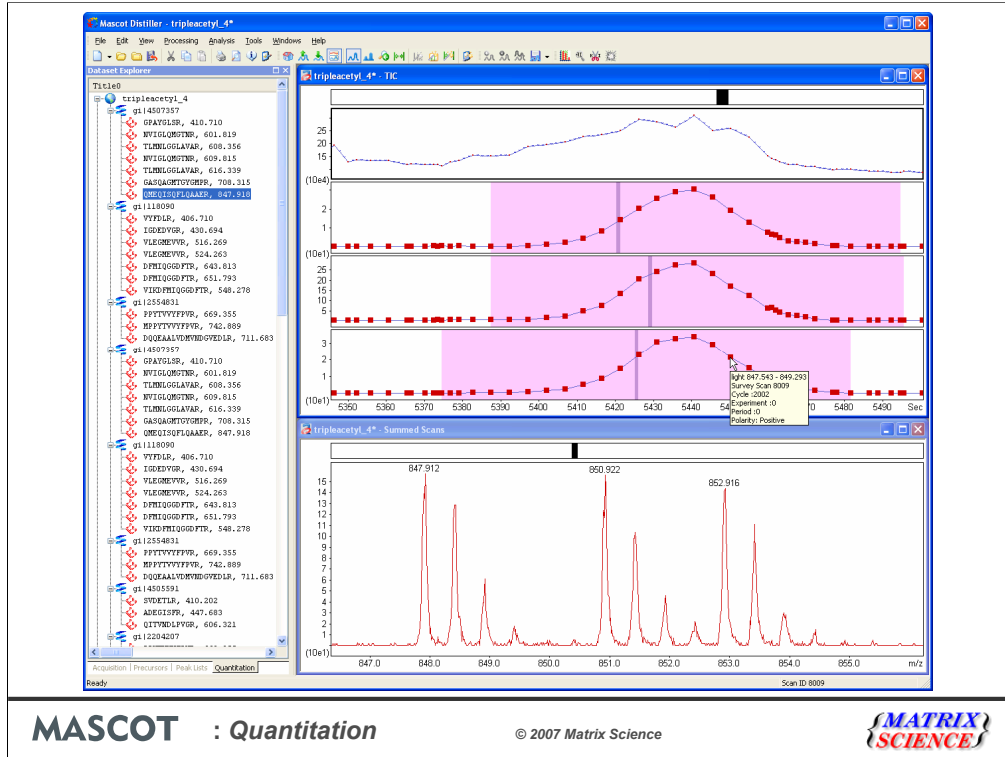
Peak detection is tricky for reporter ions. The peaks do not have a natural isotope distribution

- Turn off de-isotoping
- Don't use smart peak detection, like Mascot Distiller

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Exp	Ion	Mod	Protein		
<input checked="" type="checkbox"/> 54	534.7767	1067.5388	1067.5491	-0.0103	0	38				K.NAQEQQQQLK.S		
<input checked="" type="checkbox"/> 96	625.8919	1249.7692	1249.7638	0.0055	0	36				R.LENQLPQGAQ.V		
<input checked="" type="checkbox"/> 114	661.8789	1321.7432	1321.7597	-0.0165	0	34				K.AASGDLIIDLGSITLTF		
<input checked="" type="checkbox"/> 118	670.3865	1338.7584	1338.7387	0.0198	0	(19)				R.GDADFVDIHTSFAMGT		
<input checked="" type="checkbox"/> 119	670.3867	1338.7588	1338.7387	0.0202	0	57	0			R.GDADFVDIHTSFAMGT		
<input checked="" type="checkbox"/> 134	705.4144	1408.8142	1408.7805	0.0337	0	68	0.0			R.GDADFVDIHTSFAMGT		
<input checked="" type="checkbox"/> 143	723.4282	1444.8418	1444.8282	0.0137	0	35				R.GDADFVDIHTSFAMGT		
<input checked="" type="checkbox"/> 147	751.8827	1501.7508	1501.8092	-0.0584	0	18	17	1		R.GDADFVDIHTSFAMGT		
<input checked="" type="checkbox"/> 148	757.9334	1513.8522	1513.8344	0.0179	0	42	0.077	1	3.981	4.020	6.726	R.LENQLPQGAQ.V
<input checked="" type="checkbox"/> 166	1119.1100	2236.2054	2236.2550	-0.0504	0	61	0.00089	1	1.773	7.411	11.119	K.AASGDLIIDLGSITLTF
<input checked="" type="checkbox"/> 172	789.3806	2365.1200	2365.1495	-0.0295	0	70	6.7e-05	1	1.121	0.964	2.058	R.GDADFVDIHTSFAMGT
<input checked="" type="checkbox"/> 173	789.3896	2365.1470	2365.1495	-0.0025	0	(29)	1.1	1	0.995	1.113	1.314	R.GDADFVDIHTSFAMGT
<input checked="" type="checkbox"/> 174	789.3903	2365.1491	2365.1495	-0.0004	0	(3)	3.8e+02	1				R.GDADFVDIHTSFAMGT
<input checked="" type="checkbox"/> 175	789.7163	2366.1271	2365.1495	0.9776	0	(10)	81	1				R.GDADFVDIHTSFAMGT
<input checked="" type="checkbox"/> 222	1028.8178	3083.4316	3083.4572	-0.0257	0	(17)	7.8	1				R.CGDVDFYPNGPSTGVPGS
<input checked="" type="checkbox"/> 223	1029.1577	3084.4513	3083.4572	0.9940	0	25	1.5	1				R.CGDVDFYPNGPSTGVPGS
<input checked="" type="checkbox"/> 224	1090.1604	3267.4594	3267.5411	-0.0817	1	9	27	1				R.CGDVDFYPNGPSTGVPGS
<input checked="" type="checkbox"/> 229	1294.9831	3881.9275	3882.0043	-0.0768	1	(21)	4	1	1.261	0.026	0.037	K.WLTATELENVPSLMDITW
<input checked="" type="checkbox"/> 230	971.4973	3881.9601	3882.0043	-0.0442	1	22	3.4	1				K.WLTATELENVPSLMDITW

MASCOT : Quantitation © 2007 Matrix Science

If you wish, you can display ratios for individual peptides. The reason there is no standard deviation for the 117/114 ratio is that it failed the normal distribution test. As you can see, the individual peptide ratios have quite a scatter. One thing you have to be very careful with is peak detection. Reporter ions do not have a natural isotope distribution, so anything that expects this, like Mascot Distiller, will not be reliable. Definitely advisable to experiment with the peak picking conditions.



Finally, I can reassure you that we are hard at work on the final piece of the jigsaw, the Quantitation Toolbox for Mascot Distiller.