

Multi-Q Web Server

User Guide

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Last updated 2/16/2007

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I. Introduction

The iTRAQ labeling method combined with shotgun proteomic techniques represents a new dimension in multiplexed protein quantitation for relative protein expression in different cell states. However, the analysis of high-throughput spectral data becomes intractable. Therefore, we presented an automated tool called Multi-Q, which is designed as a generic platform that can accommodate various input data formats from search engines and mass spectrometer manufacturers. To ensure more accurate quantitation results and to reduce manual validation efforts, in the design of Multi-Q, we paid particular attention to the following issues. First, iTRAQ's relatively large sample complexity inevitably includes identical peptides produced by two homologous proteins. These degenerate (non-unique) peptides not only result in ambiguous protein identification, but also introduce protein quantitation errors. Multi-Q quantitation is based on the nondegenerate peptides. Second, instrument's dynamic range of detection will very likely affect quantitation results. Thus, Multi-Q is designed to allow users to input the threshold for dynamic range filtering. This work has been published in *J Proteome Research* **5** (9), 2328-2338, 2006, and a stand-alone program executable on Windows platform has been available for download.

Now, we present an improved version of Multi-Q, called Multi-Q Web Server. In comparison with its previous stand-alone version, Multi-Q Web Server provides many enhanced features and flexible options for quantitation. The use of the web server is guided by a quantitation wizard so that the tool can be easy to use. Furthermore, it provides friendly user interface for output. The web server outputs a default report for quantitation results. In addition, it allows users to customize their output report and information of user's interest can be easily highlighted. The output provides visualization of mass spectral data so that users can conveniently validate the results. Multi-Q Web Server is a fully automated, friendly and easy to use quantitation tool suitable for large-scale multiplexed protein quantitation.

II. Multi-Q Web Server Workflow

Multi-Q Web Server accepts spectral data and search results from MASCOT and SEQUEST as input data and, in particular, provides a program called *mzFast* to convert WIFF raw spectral data into a reduced *mzXML* file so that the conversion time can be much reduced. When the web server accepts *mzXML* files converted from RAW and BAF files directly as input, it automatically determines to read the reduced *mzXML* files so that the loading time can also be reduced. Then the main program of the web server calculates peptide relative expression levels (peptide ratios) and protein relative expression levels (protein ratios). Finally, the web server outputs both quantitation results and the identities of quantified peptides and proteins.

The workflow of Multi-Q is depicted in the following figure.

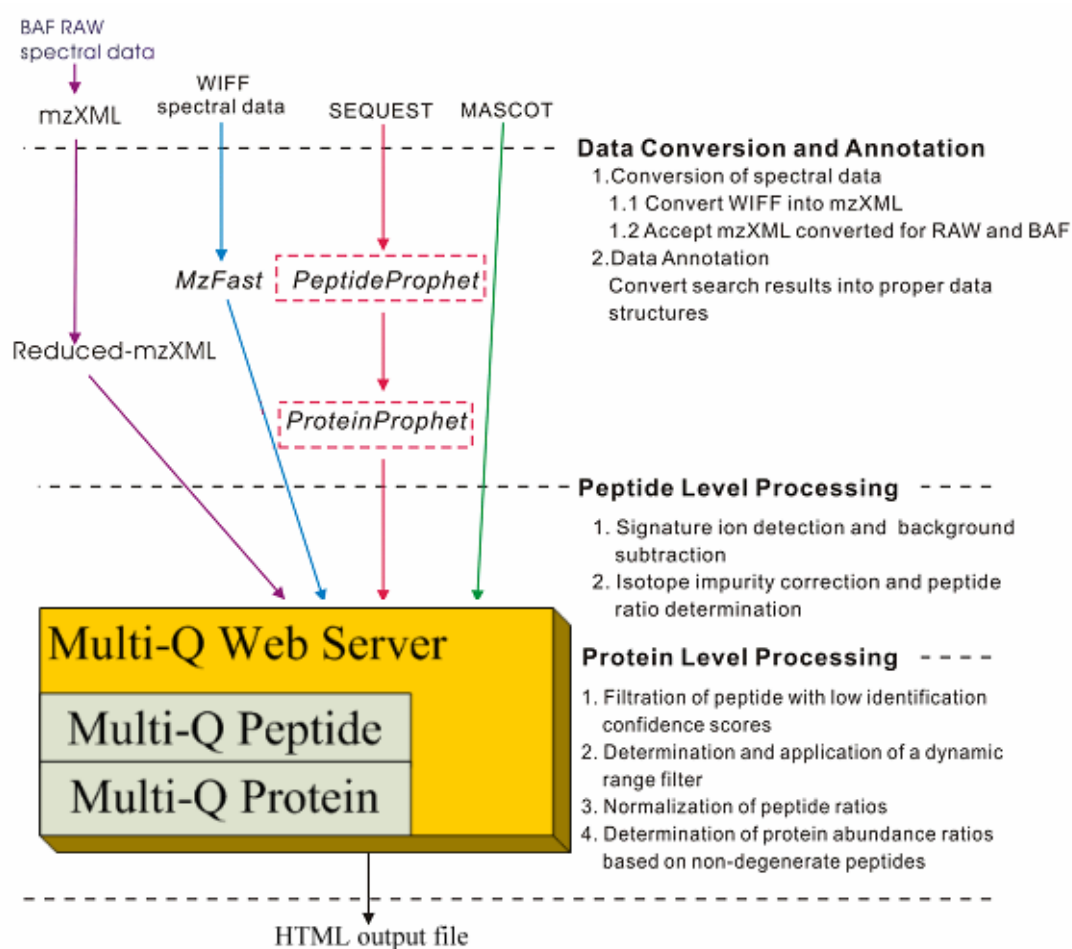


Figure 1: Multi-Q Web Server workflow

III. System Requirements and Download

1. System Requirements

1.1. Server

1.1.1. Multi-Q Web Server runs on the Windows Server 2003 (recommended), Windows XP Professional, and Windows 2000, and requires Microsoft .NET framework version 2.0 (or higher) available at <http://www.microsoft.com/net/>.

1.2. Data Preparation

1.2.1. Analyst QS® from <http://www.appliedbiosystems.com/index.cfm>. (Required only when using mzFast converter.)

1.2.2. Mascot script (Mascot.dll) for Analyst QS from http://www.matrixscience.com/help/instruments_analyst.html: This script provides a convenient interface to Mascot. (Required only when using Mascot to search .WIFF files.)

1.2.3. The MSQuant tool, MultRawPrepare: It can combine several raw spectral files and can be found at <http://msquant.sourceforge.net/> or <http://www.pil.sdu.dk/1/MSQuant/MultRawPrepare,2004-11-12.zip>. (Required only when you need to combine multiple spectral files into one file.)

1.3. End Users

1.3.1. Microsoft Internet Explorer version 6.0 (or higher).

2. Software Download

Users can download “Multi-Q Web Server Installation” from our website <http://ms.iis.sinica.edu.tw/Multi-Q-Web> and unzip it to a user-defined directory using an unzip software, such as WinZip® or 7-Zip (free software). After unzipping, users will find the following directories and files:

- 2.1. bin (directory):** This directory contains essential libraries of Multi-Q Web Server.
- 2.2. Experiment_Data (directory):** Multi-Q Web Server considers this directory as the running root folder. Users need to put input data files in this directory.
- 2.3. Tools (directory):** This directory contains miscellaneous tools including mzFast and wiff2Scan.
- 2.4. ZedGraphImages (directory):** This directory stores real-time generated charts, such as mass spectrum charts.
- 2.5. Main programs and configuration files (files):** Main programs of Multi-Q Web Server, such as .aspx files, and their configuration files are also included in the ZIP file.

IV. Input Data Preparation

Multi-Q Web Server requires spectral data files (.mzXML) and search results as input data files. Multi-Q Web Server sets the Experiment_Data folder as the root folder. We recommend users create a folder under the root to store all input files (including the search result file, spectral files, and .table file) of one experiment.

1. Preparing Spectral Input Files

Users having spectral data of BAF and RAW formats need to obtain mzXML files as input files. For users having WIFF spectral files, Multi-Q Web Server provides a program called mzFast to generate mzXML files.

➤ The mzFast Converter

The mzFast program converts WIFF spectral files into “reduced” mzXML files. Since mzFast uses a Visual Basic library provided by the Analyst Software, users need to install Analyst QS® on their computers.

There are two different modes in mzFast: basic mode and batch mode.

- Basic Mode

The basic mode converts a WIFF file one at a time as shown left below.

1. mzFast can be switched to batch mode by clicking on this button.
2. Input a WIFF file (spectral raw data file) by clicking “Browse”.
3. By clicking “Browse”, users can select a directory to contain the output mzXML file; users are required to enter the full name of the output file.
4. By checking the box and entering the cutoff m/z value, the program can generate a “reduced” mzXML file. The default cutoff value is 120.
5. The box of Instrument allows users to select their experiment configuration type.
6. Users can set peak filtration condition by entering the cutoff peak intensity value. Peaks with intensity lower than this value will be discarded. The default filtration threshold is 0.

- Batch Mode

The batch mode allows users to convert multiple data files at a time as shown right below.

1. Input WIFF files by selecting a directory in the leftmost window. mzFast will automatically include all WIFF files in the directory and show them in the middle window.
2. mzFast can be switched to basic mode by clicking on the button.
3. Perform the same steps as Steps 4-6 of Basic mode.

Finally, users click the “Convert” button to generate the reduced mzXML file(s). For batch mode, the generated files will be shown in the rightmost window of Step 1.

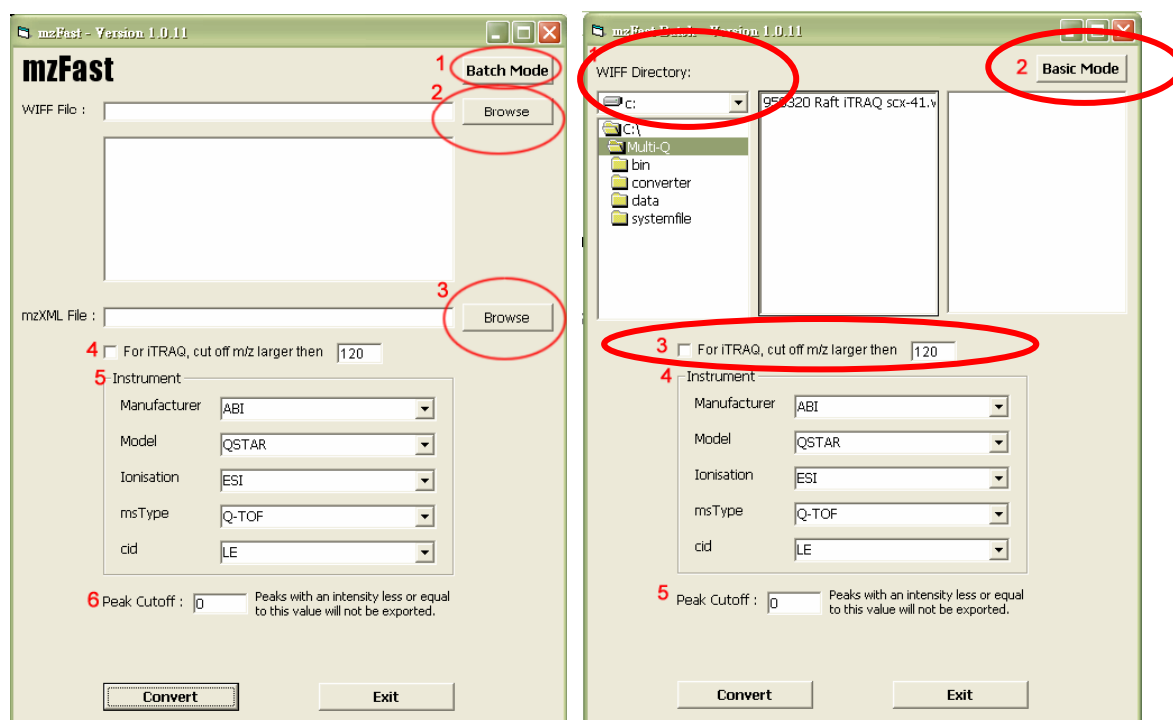


Figure: mzFast - Basic Mode and Batch Mode

2. Preparing Search Results

Multi-Q Web Server accepts search results from Mascot and SEQUEST.

2.1 For Mascot Users

Data preparation of Mascot users is shown in the following diagram.

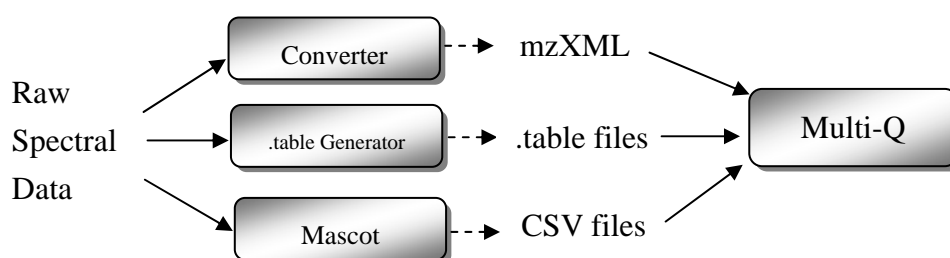


Figure: Input data for Mascot users

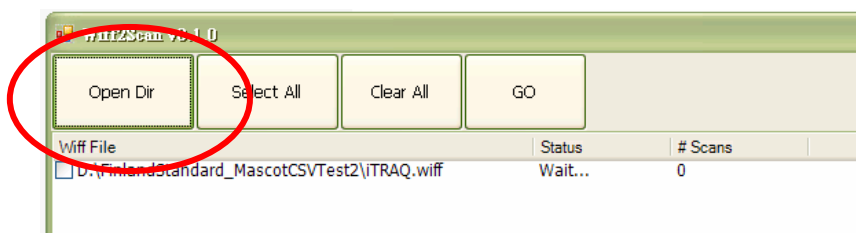
For Mascot users, Multi-Q Web Server requires three types of files as input: mzXML files, .table files and CSV files. Users can convert raw spectral data to mzXML files as previously mentioned. In Mascot CSV files, peptides are indexed by query number, which has its corresponding spectrum information. Since quantitation is based on the scan number, we have developed a wiff2scan program to map the query number to the scan number. Mascot users need to run wiff2scan first to generate the mapping file (.table file). Finally, after exporting CSV files from Mascot, users can use Multi-Q Web Server to quantify the input

files of their experiments.

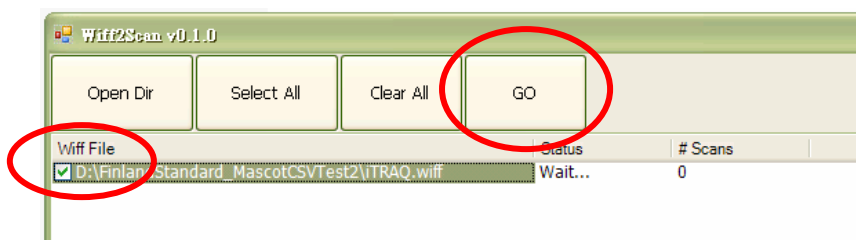
2.1.1 .table Generator

The wiff2scan program generates .table files for WIFF spectral files. The procedure to use wiff2Scan is given below.

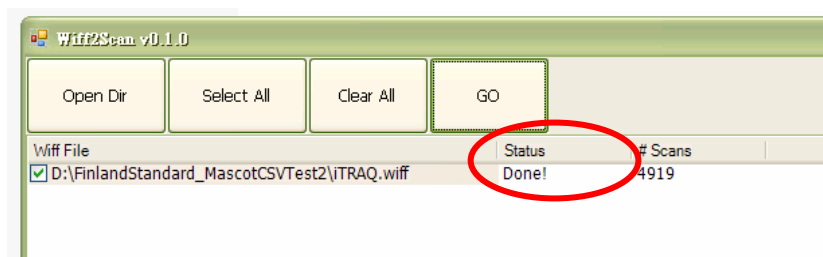
- i 、 Click “Open Dir” button and select the directory where .WIFF files locate. Then you will see all WIFF files in the folder (as shown below).



- ii 、 Select WIFF files by checking the boxes of the files, and click “GO” button (as shown below).



- iii 、 When the process is completed, you will see “Done!” in the “status” field and .table files in the folder (as shown below).



2.1.2 Using Analyst QS® and Mascot to Search for One .WIFF File

Though Mascot can accept many types of data as input, Multi-Q Web Server currently equips with the capability for users having WIFF spectral files to conveniently generate Mascot search results (CSV file). Users having only one WIFF file can follow the procedure below.

- i 、 Install Mascot script for Analyst QS®. You can find installation details in

http://www.matrixscience.com/help/instruments_analyst.html. “Mascot.dll” will appear in the “script” of the Analyst QS® (as shown below).

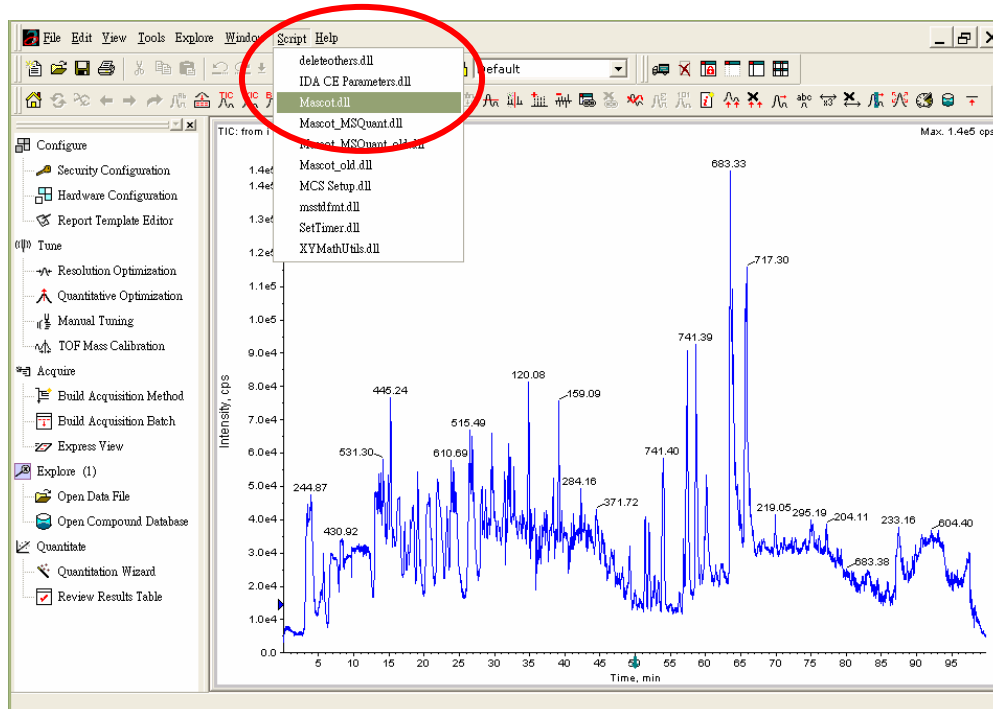


Figure: Mascot script in the Analyst QS

- ii · Open the .WIFF file with Analyst QS® and select Mascot script (“Mascot.dll”).
- iii · Users will see a pop-up “MASCOT MS/MS ions search” form and can set their parameters for search. Then click “Start Search” button to search the .WIFF file.

MASCOT MS/MS Ions Search

Your name	Yian	Email	m2k.m2k@mail2000.com.tw
Search title	D:\FinlandStandard_MascotCSVTest2\iTRAQ.wiff (sample number 1)		
Database	IPI_human		
Taxonomy	All entries		
Enzyme	Trypsin	Allow up to	2 missed cleavages
Fixed modifications	Acetyl (K) Acetyl (N-term) Amide (C-term) Biotin (K) Biotin (N-term)	Variable modifications	EtOH (C) Guanidination (K) HNE (CHK) HSe (C-term M) Hse_lact (C-term M)
Protein mass	kDa		
Peptide tol. ±	0.7 Da	MS/MS tol. ±	0.3 Da
Peptide charge	2+		
Data file	I:\LOCALS~1\Temp\mas2D.tmp		
Data format	Mascot generic	Precursor	m/z
Instrument	ESI-QUAD-TOF		
Overview	Report top		AUTO hits
Start Search ...		Reset Form	

Figure: Mascot MS/MS ions search interface after executing Mascot script

2.1.3 Using Analyst QS® and Mascot to Search for Multiple .WIFF

Files

Multi-Q Web Server accepts only a single file of search results as input. When users have multiple WIFF files, they can obtain the search result file by either combining multiple search results or searching the integrated spectral data file. We suggest the latter since the search results can better reflect proteins contained in the experiment. Tools for combining multiple WIFF files into one file are available at the world wide web. Procedure to search the combined file using the MSQuant tool is introduced below.

- i 、 Perform Steps i and ii in Section 2.1.2.
- ii 、 Retrieve the output file of Analyst QS® and change the file name: Similar to Step iii in 2.1.2, a “MASCOT MS/MS ions search” form will pop up. Obtain the file listed in the “Data file” field as shown below (Usually in [C:\Documents and Settings\USER\Local Settings\Temp](#)) and copy it to the folder where the spectral files locate. Change the file name to RawDataFileName + “.msm”, for example, you can change the file name to “iTRAQ_1.msm” if you have an iTRAQ_1.wiff file.

MASCOT MS/MS Ions Search

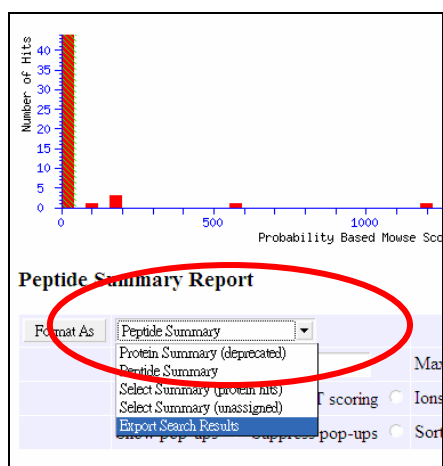
Your name	<input type="text" value="Yian"/>	Email	<input type="text" value="m2k.m2k@mail2000.com.tw"/>
Search title	<input type="text" value="D:\FinlandStandard_MascotCSVTest2\iTRAQ.wiff (sample number 1)"/>		
Database	<input type="text" value="IPI_human"/>		
Taxonomy	<input type="text" value="All entries"/>		
Enzyme	<input type="text" value="Trypsin"/>	Allow up to	<input type="text" value="2"/> missed cleavages
Fixed modifications	<input type="text" value="Acetyl (K)"/> <input type="text" value="Acetyl (N-term)"/> <input type="text" value="Amide (C-term)"/> <input type="text" value="Biotin (K)"/> <input type="text" value="Biotin (N-term)"/>	Variable modifications	<input type="text" value="EtOH (C)"/> <input type="text" value="Guanidination (K)"/> <input type="text" value="HNE (CHK)"/> <input type="text" value="Hse (C-term M)"/> <input type="text" value="Hse_lact (C-term M)"/>
Protein mass	<input type="text"/> kDa	ICAT	<input type="checkbox"/>
Peptide tol. ±	<input type="text" value="0.7"/> Da	MS/MS tol. ±	<input type="text" value="0.3"/> Da
Peptide charge	<input type="text" value="2+"/>	Monoisotopic	<input checked="" type="radio"/> Average <input type="radio"/>
Data file	<input type="text" value="I:\LOCALS~1\Temp\mas2D.tmp"/> <input type="button" value="Browse..."/>		
Data format	<input type="text" value="Mascot-generic"/>	Precursor	<input type="text"/> m/z
Instrument	<input type="text" value="ESI-QUAD-TOF"/>		
Overview	<input type="checkbox"/>	Report top	<input type="text" value="AUTO"/> hits
<input type="button" value="Start Search ..."/>		<input type="button" value="Reset Form"/>	

- iii 、 Repeat Step ii until all .WIFF files have the corresponding .msm files.
- iv 、 Download MSQuant combination tool (MultRawPrepare) and unzip it to the folder of .msm files.
- v 、 In MultRawPrepare.bat, change INDIR to the folder containing the .msm files and OUTFILE to a user-defined file name, for example, iTRAQ_all.msm.
- vi 、 Execute MultRawPrepare.bat and the user will obtain the combined msm file (e.g., iTRAQ_all.msm) in the folder.
- vii 、 Run Mascot MS/MS ions Search using the combined .msm file.

2.1.4 Exporting Mascot Search Results to CSV Files

Users can take the following steps to export CSV files:

- i. In the web page of Mascot Search Results, click the pull-down menu of “Format AS” in “Peptide Summary Report” and select “Export Search Results”, and then click the “Format AS” button, as shown below.



- ii. In the “Export search results” page, select the “CSV” format for “Export format” field. Select “Standard” in the “Protein scoring” field (shown left below), and check the box in the “Input query data” field (shown right below). Then click “Export Search Results” button at the bottom of the page. Finally, the user can save the generated CSV file.

Optional Search Information

Header	<input checked="" type="checkbox"/>
Search parameters	<input checked="" type="checkbox"/>
Format parameters	<input checked="" type="checkbox"/>
Residue masses	<input type="checkbox"/>
Unassigned queries (peptide matches not assigned to protein hits)	<input type="checkbox"/>
Input query data	<input checked="" type="checkbox"/>

Optional Protein Hit Information

2.2 For SEQUEST Users

SEQUEST user’s input to Multi-Q Web Server is shown in the diagram below.

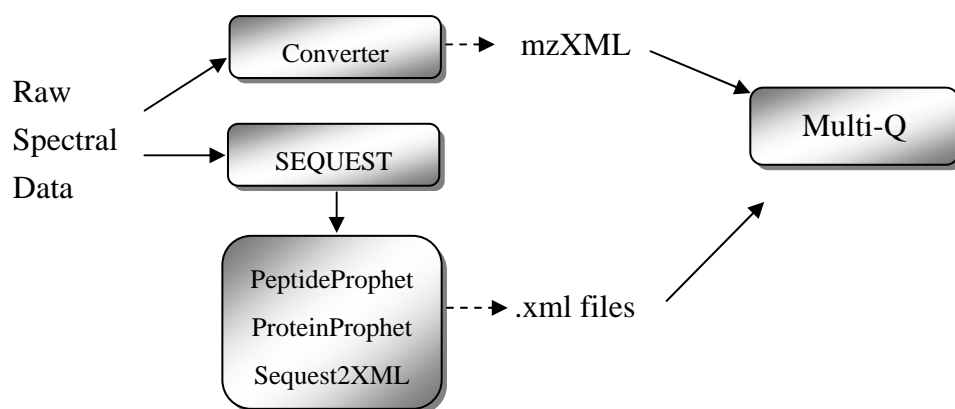


Figure: Input data for SEQUEST users

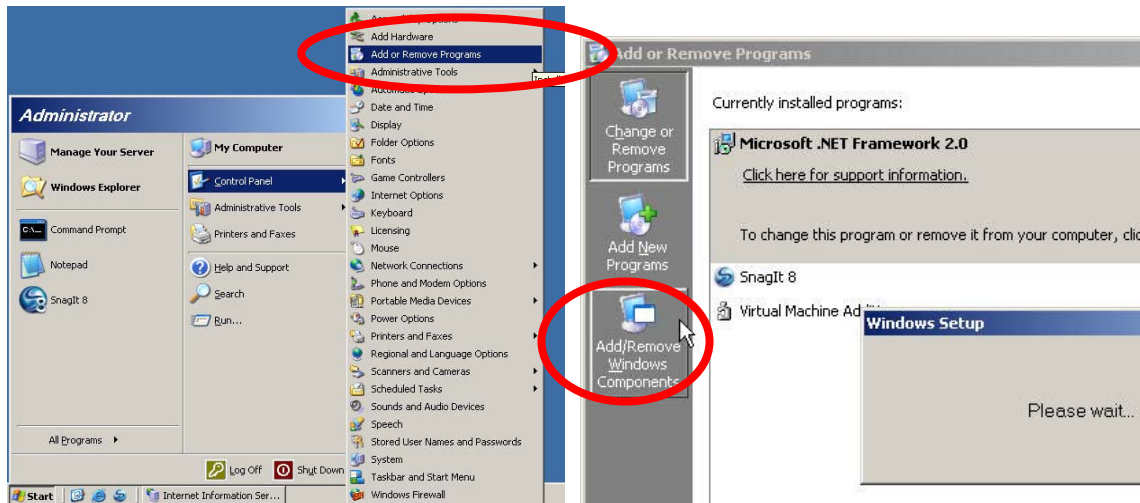
For SEQUEST users, Multi-Q Web Server requires mzXML files and validated SEQUEST search results (.xml) as input. That is, users need to apply PeptideProphet and ProteinProphet on the search results before they input the results into Multi-Q Web Server.

V. Installing Multi-Q Web Server

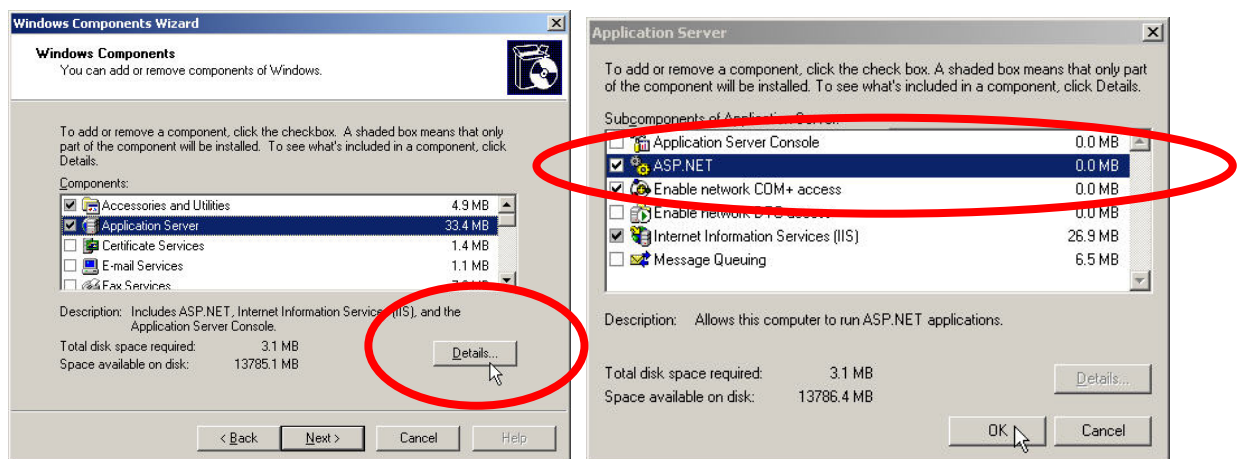
Users need to install Microsoft .NET Framework 2.0 or higher in the server machine before installing Multi-Q Web Server. We illustrate the installation procedure in Windows Server 2003 below. (The screenshot may be slightly different depending on the operation system and environment settings.)

1 Installing the Internet Information Service (IIS) Web Server and ASP.NET

- 1.1 Select “Start”→“Control Panel”→”Add or Remove Programs” (shown left below).
- 1.2 Press “Add/Remove Windows Components” button (shown right below).



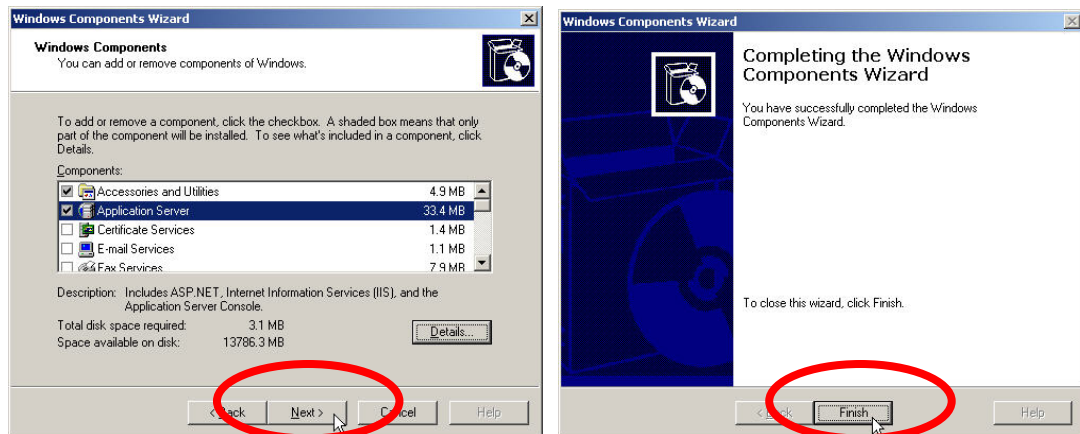
- 1.3 Select “Application Server” and press the “Details” button in Windows Components Wizard (shown left below).
- 1.4 Check the “ASP.NET” box. Then “Enable network COM+ access” and “Internet Information Services (IIS)” will be automatically checked. Press the “OK” button (shown right below).



- 1.5 Press “Next” button in “Windows Components Wizard”, and installation will

automatically start (shown left below).

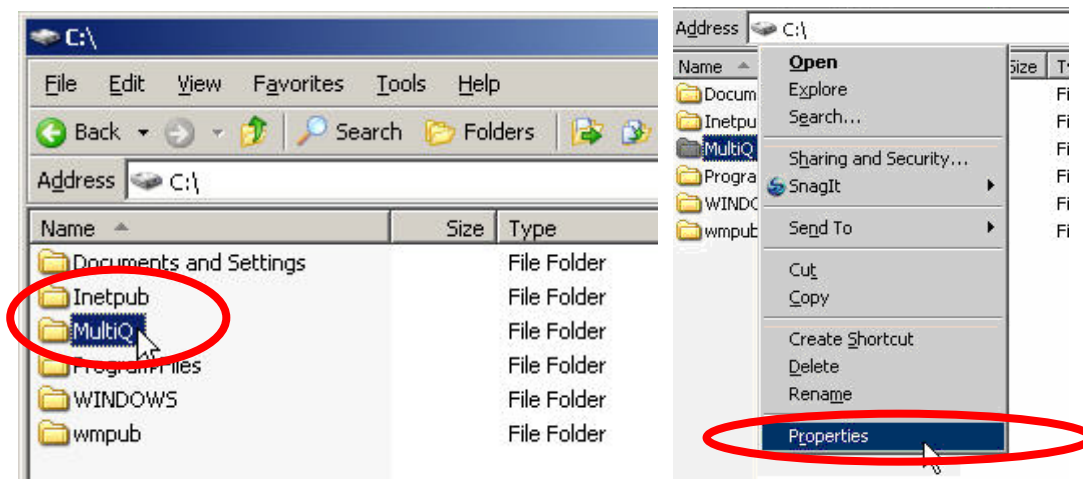
- 1.6 Press the “Finish” button when installation is completed (shown right below).



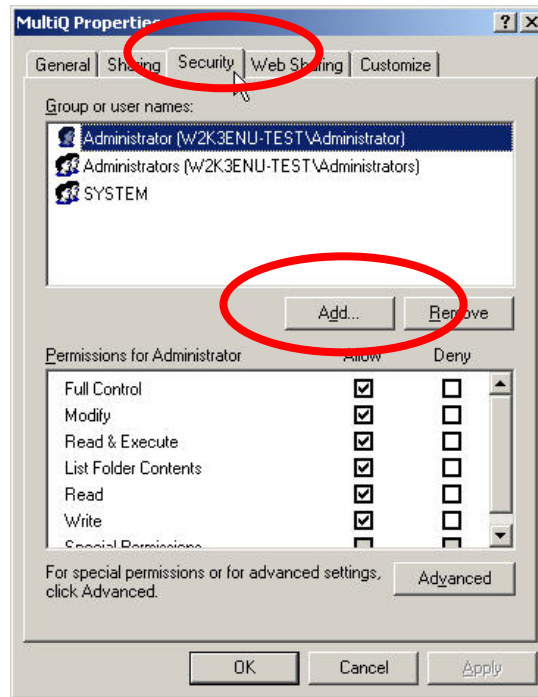
2 Post-Installation Configuration

Users can take the following steps to create Administrators.

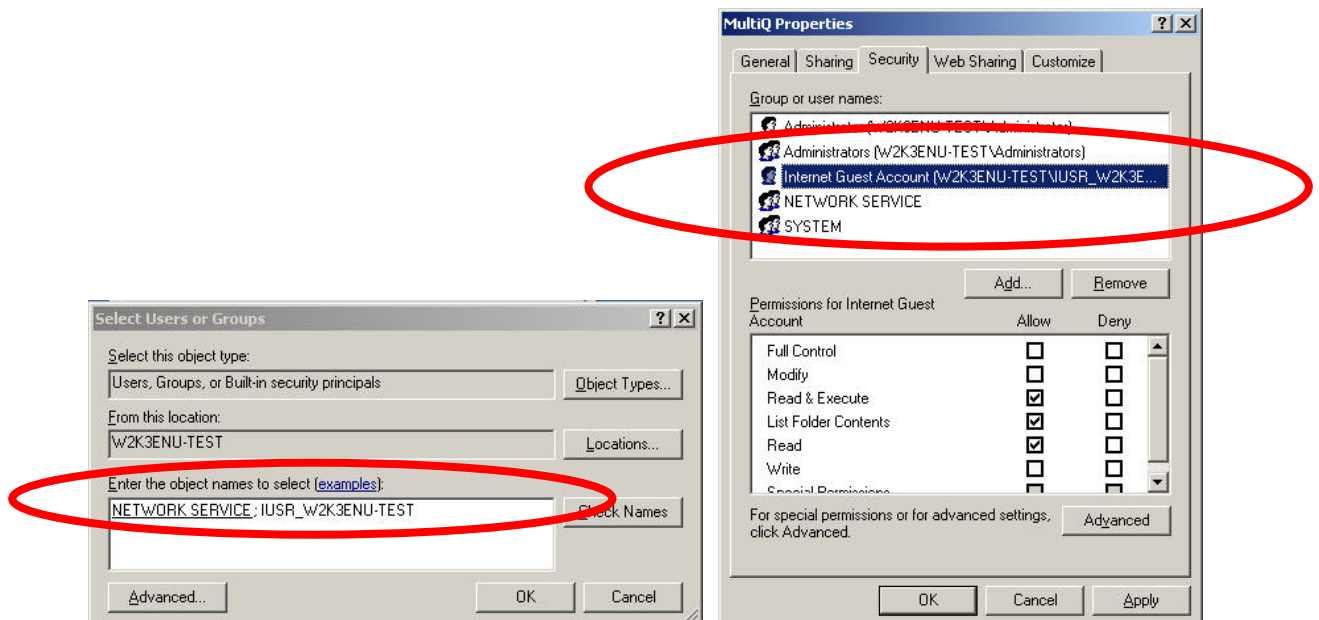
- 2.1 Unzip “Multi-Q Website Install File.zip” to your favorite directory, such as “C:\”, and rename the folder, e.g., “MultiQ” (shown left below).
- 2.2 Select the folder you extracted and renamed, and right-click the folder and select “Properties” (shown right below).



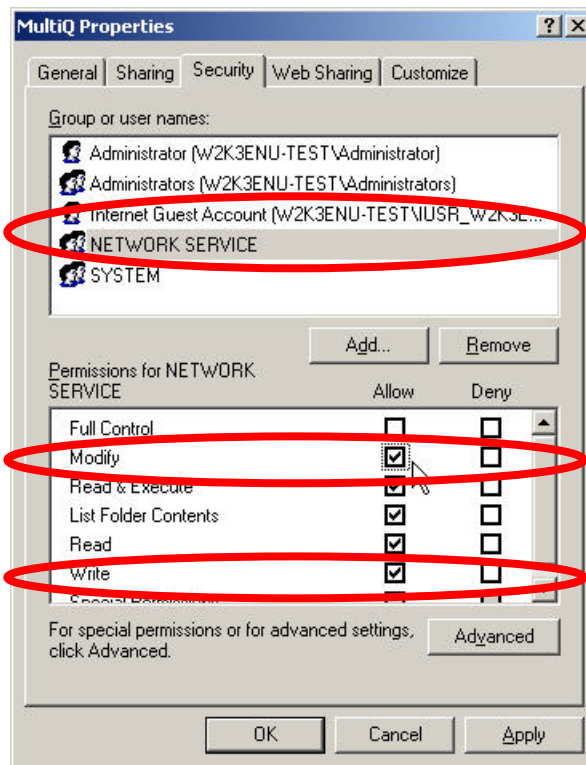
- 2.3 Select “Security” tab in “MultiQ Properties” form and click the “Add” button.



- 2.4 In “Select Users or Groups” form, type “Network Service; IUSR_<computer name>” in the “Enter the object names to select” text box, where <computer name> is the server’s name, e.g., “W2K3EUI-TEST” for our server. Then click the “OK” button, and “Internet Guest Account” and one group “NETWORK SERVICE” are added in the “Group or user names” list box.



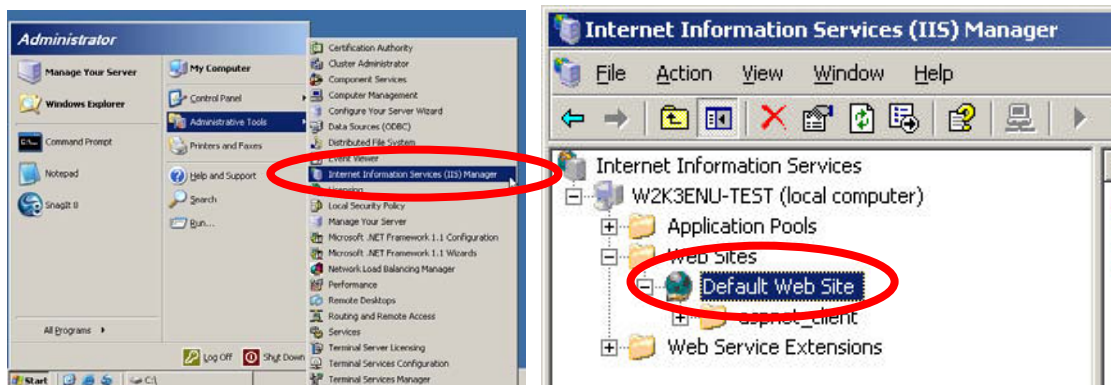
- 2.5 Select “NETWORK SERVICE” and check “Modify” and “Write” in the “Allow” column in “Permissions for NETWORK SERVICE”. Finally, click the “OK” button.



3 Constructing Multi-Q Web Server in Internet Information Services

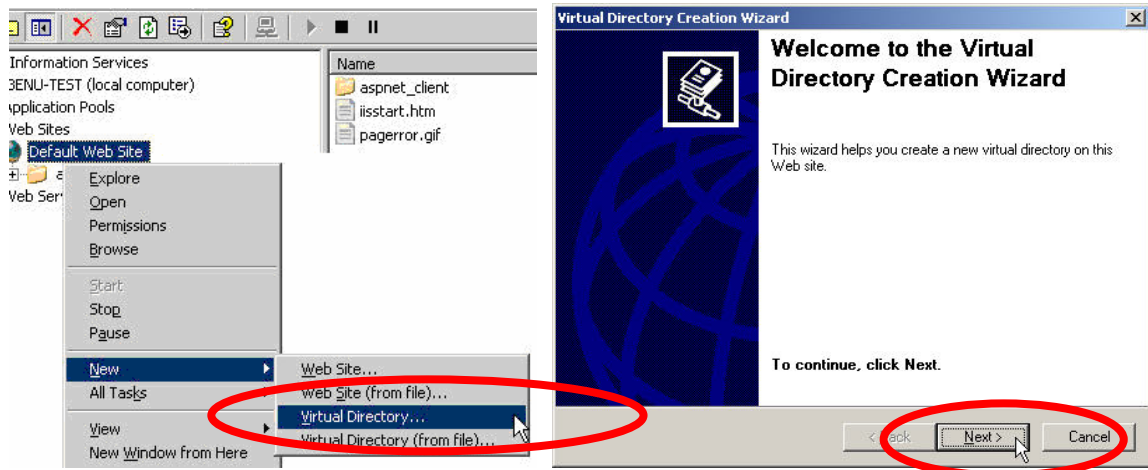
3.1 Open “Start”→” Administrative Tools”→”Internet Information Services (IIS) Manager” (shown left below).

3.2 Select “Default Web Site” in the “Web Sites” node (shown right below).



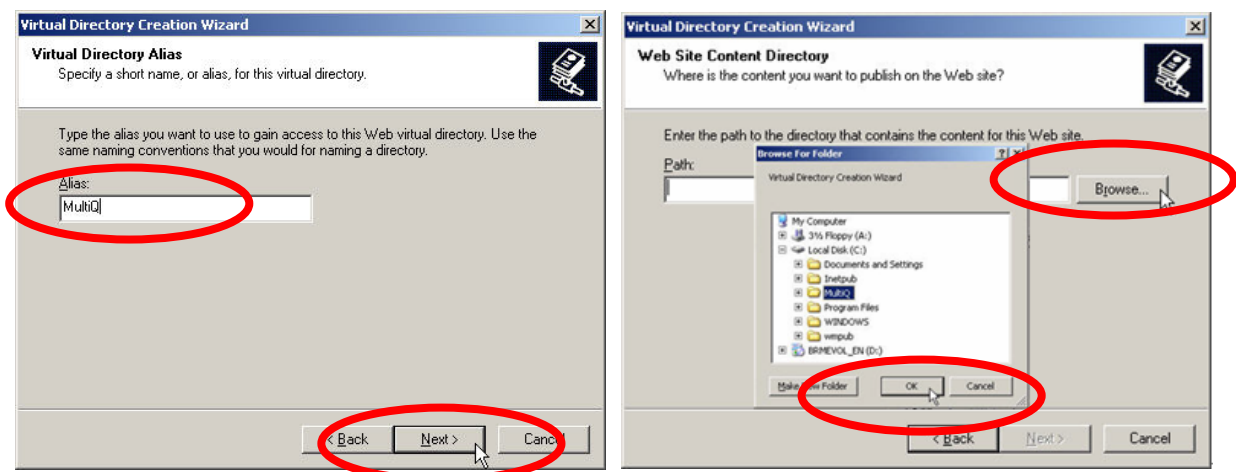
3.3 Right-click “Default Web Site”→”New”→Select” Virtual Directory” (shown left below).

3.4 Click the “Next” button in “Virtual Directory Creation Wizard” (shown right below).



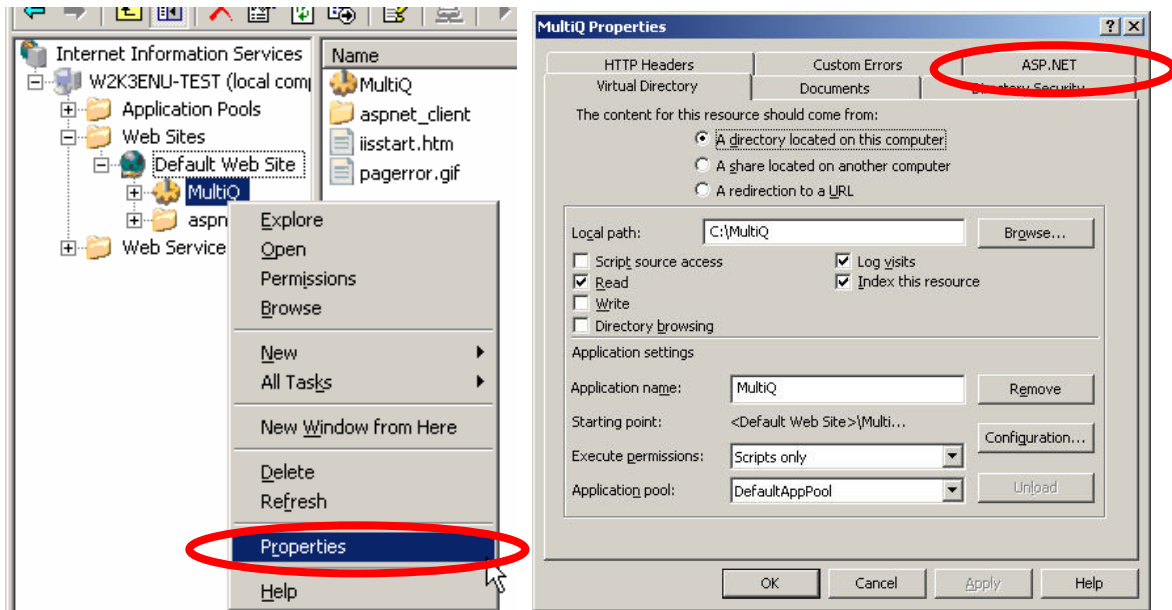
3.5 Key in “MultiQ” in Alias text box and click “Next” button (shown left below).

3.6 Click the “Browse...” button in the “Web Site Content Directory” form, and select “MultiQ” in the pop-up “Browse for Folder” window and click the “OK” button (shown right below).

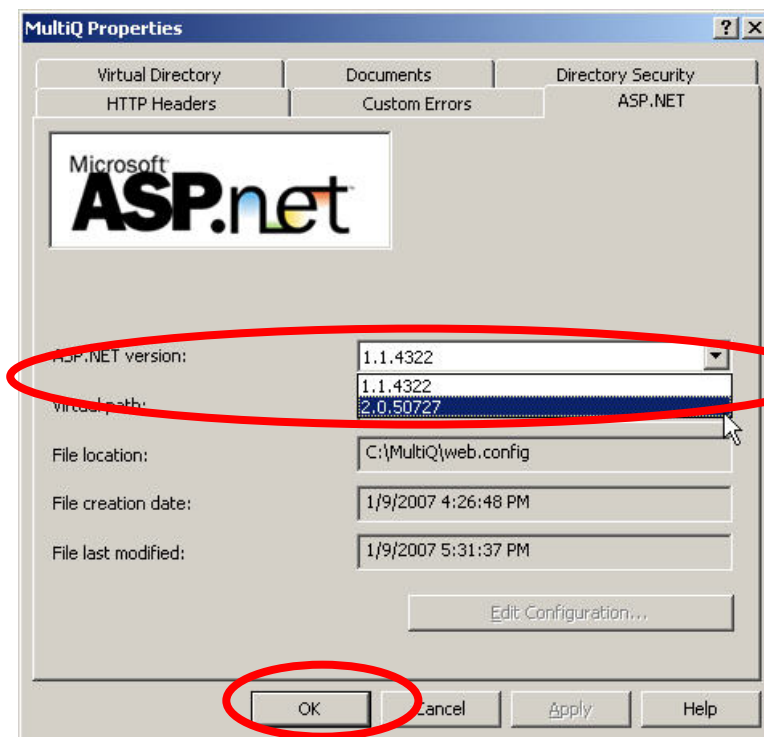


3.7 Right-Click “MultiQ” and select “Properties” (shown left below).

3.8 Select “ASP.NET” tab in “MultiQ Properties” form (shown right below).



3.9 Select “2.0.50727” or higher in “ASP.NET version” field, and click the “OK” button in the “MultiQ Properties” form.

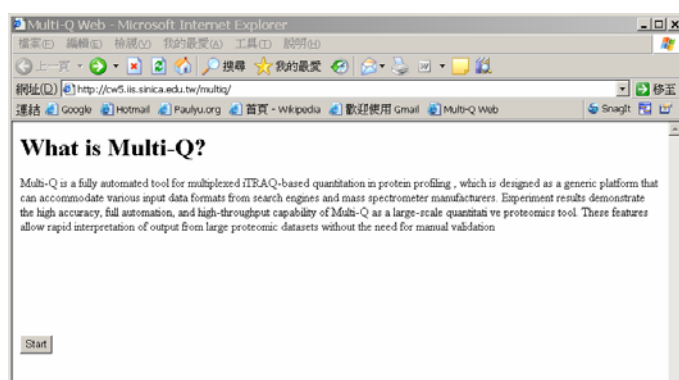


VI. Running Multi-Q

Before quantitation, all input data should be ready in the “Experiment_Data” folder.

Procedure to run Multi-Q is described below.

- 1 Open Microsoft Internet Explorer and key in the URL “http://servername/Multiq/default.aspx”. (“servername” is the domain name or IP of the server, e.g., “W2K3EU-TEST” in our example.) Then click the “Start” button (shown left below), and the “Multi-Q Quantitation Wizard” window will pop up, and click “Start” in the wizard (shown right below).



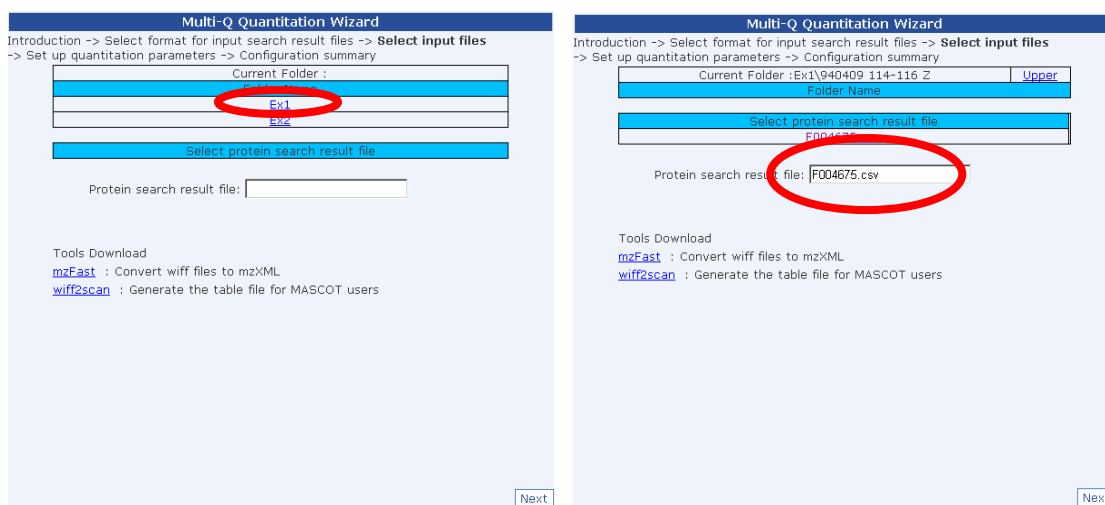
- 2 Select the search result type.



- 3 Select the input files.

For Mascot users

- i. Multi-Q Web Server will list files and directories in “Experimental_Data” folder, e.g., “Ex1” and “Ex2” in our example (shown left below).
- ii. Select the folder name that contains the input data, i.e., the search result file, mzXML files and .table file (shown left below).
- iii. Click the file name in “Select protein search result file” field, and the file name will automatically appear in the text box (shown right below). (Note: When Multi-Q performs quantitation, it will automatically retrieve other input files, including mzXML files and .table file.)



For SEQUEST users:

- i. Multi-Q Web Server version would list files and directories in “Experimental_Data” folder, such as “Ex1” and “Ex2” in our example..
- ii. Select the folder name that contains input data, i.e., the search result files from ProteinProphet and PeptideProphet, mzXML files. (shown left below)
- iii. Click the file name in “Select protein search result file” field, and the file name will automatically fill into the “protein search result file”.

Click the file name in “Select peptide search result file” field, and the file names will automatically appear in the “peptide search result file”. (The right-below figure shows the results after Steps iii and iv.) Then click the “Next” button.

Multi-Q Quantitation Wizard
Introduction -> Select format for input search result files -> **Select input files**
-> Set up quantitation parameters -> Configuration summary

Current Folder :
Folder Name
Ex2

Select protein search result file: 950502_12105.xml
Select peptide search result file: interact-pep.xml

Protein search result file: interact-prot.xml
Peptide search result file: interact-pep.xml

Tools Download
mzFast : Convert wiff files to mzXML
wiff2scan : Generate the table file for MASCOT users

Next

4 Setting up quantitation parameters.

Multi-Q Quantitation Wizard
Introduction -> Select format for input search result files -> Select input files
-> **Set up quantitation parameters** -> Configuration summary

Sample/standard data type Sample Standard
Sample:normalization function will be performed
Standard:normalization function will not be performed

Isotope Impurity Correction Table

Reagent	% of -2	% of -1	% of +1	% of +2
iTRAQ Reagent 114	0.0	1.0	5.9	0.2
iTRAQ Reagent 115	0.0	2.0	5.6	0.1
iTRAQ Reagent 116	0.0	3.0	4.5	0.1
iTRAQ Reagent 117	0.1	4.0	3.5	0.1

Configuration

Perform curve smoothing and background subtraction
 Use confident peptides score > 0.0 for protein quantitation
 Use only nondegenerate peptides for protein quantitation
 Weighted calculation to determine protein ratios
 Dynamic Range

Low Intensity 0.0
High Intensity 0.0

	Ratio 1(R1)	Ratio 2(R2)	Ratio 3(R3)
Numerator	m/z 115	m/z 116	m/z 117
Denominator	m/z 114	m/z 114	m/z 114

Next

The configuration page provides different data filtering options that allow users to define the following kinds of thresholds for customizing their quantitation.

4.1 Sample/standard data type: Multi-Q Web Server version would apply normalization function for the sample data and ignore normalization file for the standard data.

First-time users of Multi-Q are recommended to run a set of standard data to determine the dynamic range of their instruments.

- 4.2 Software Correction Factors: Users can input their own isotope correction values into the table.
- 4.3 Users can select whether or not to “Perform smoothing and background subtraction”.
- 4.4 Peptide Score: Users can determine the peptide score threshold to filter out peptides with low identification confidence.
- 4.5 Non-Degenerate Peptide: This option provides the function of evaluating protein ratio using only ratios of non-degenerate peptides.
- 4.6 Dynamic Range: Users can input their own mass spectrometer’s dynamic range for peptide filtration.
- 4.7 Weighted function: The checkbox of “Weighted” allows users to calculate a protein ratio by weighted or unweighted average of peptide ratios. The checkbox is set to be checked by default, i.e., weighted average calculation is used.
- 4.8 Users can define their own numerator and denominator of ratio 1, ratio 2 and ratio 3. The default setting of ratios are shown in the above figure.

Click “Next” when setting is completed.

5 Configuration Summary

When finishing **setting up quantitation parameters**, the system will show “Configuration Summary” (shown below). After clicking the “Finish” button, then the program starts quantitation calculation.

Multi-Q Quantitation Wizard

Introduction -> Select format for input search result files -> Select input files
 -> Set up quantitation parameters -> **Configuration summary**

Configuration Summary	
Quantitation name	Config
Normalization	Applied
Nondegenerate peptides	Use nondegenerate peptides
Weighted calculation	Applied
Peptide score threshold >	0
Dynamic range	
High intensity	0
Low intensity	0

	Ratio 1(R1)	Ratio 2(R2)	Ratio 3(R3)
Numerator	m/z 115	m/z 116	m/z 117
Denominator	m/z 114	m/z 114	m/z 114

Missing following mzXML file:

Finish

VII. Output Reports

Multi-Q Web Server provides two types of output reports: default output report and customized output report. The Web Server will automatically generate a default output report after users click the “Finish” button in the quantitation wizard. The system also allows users to customize their output reports. With the customized output report, users can conveniently highlight information of their interests. Users can search peptides or proteins of the output reports by using their own browser and save the reports in html format.

1. Default Output Reports

The default output report contains the following information.

1.1 Protein Summary List

Protein list shows information of all identified proteins in one experiment. Each protein, listed by its accession number (Ace#), reports its ProteinPhrophet score (Score), protein mass (Mass), protein ratios (R1, R2, R3) and their standard deviations (Std 1, Std 2, Std 3), and protein description (Desc) as shown below.

Output composition

Statistics of experiment results

Ratio 1(R1)	Ratio 2(R2)	Ratio 3(R3)
m/z 115	m/z 116	m/z 117
m/z 114	m/z 114	m/z 114

	No. of proteins with ratio 0	No. of proteins with rational-value ratios	Normalization Factor
R1	36 (36/115)	79 (79/115)	-0.001
R2	30 (30/115)	85 (85/115)	1.185
R3	32 (32/115)	83 (83/115)	0.013
	No. of proteins with std not available	No. of proteins with std 0	No. of proteins with rational-value std
Std 1	34 (34/115)	36 (36/115)	45 (45/115)
Std 2	27 (27/115)	30 (30/115)	58 (58/115)
Std 3	31 (31/115)	32 (32/115)	52 (52/115)

Protein List : 115

Acc#	Score	Mass	R1	R2	R3	Std 1	Std 2	Std 3	Desc.
IMB1 HUMAN	158	97108	0.03	1.29	0.02	N/A	0.05	0.03	(Q14974) Importin beta-1 subunit (Karyopherin beta-1 subunit) (Nuclear factor P97) (Importin 90)
K22O HUMAN	49	65830	0.01	1.24	0.02	0.00	0.08	0.01	(Q01546) Keratin, type II cytoskeletal 20a (Cytokeratin 20) (K20) (CK 20)

1.2. Peptide Summary List

By double-clicking the accession number of a protein in the Protein List, the system will report all identified peptides belonging to the protein in a new pop-up window (shown below).

IMB1_HUMAN

	No. of peptides with ratio not available	No. of peptides with ratio 0	No. of peptides with rational-value ratio	No. of peptides with infinity ratio	Calculational No.
R1	0(0/11)	3(3/11)	8(8/11)	0(0/11)	2
R2	0(0/11)	0(0/11)	11(11/11)	0(0/11)	6
R3	0(0/11)	1(1/11)	10(10/11)	0(0/11)	6

Peptide List : 11

spec.	score	sequence	z	m/z 114	m/z 115	m/z 116	m/z 117	R1	R2	R3	Mod	L	S	U	R1	R2	R3
50	5.7	VAAGLQIK	2	326.05	0.00	406.13	8.73	0	1.25	0.03	iTRAQ (Nterm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
122	27.72	VAALQNLVK	2	498.68	15.78	659.87	6.29	0.03	1.32	0.01	iTRAQ (Nterm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
550	45.4	LAATNALLNSLEFTK	2	115.92	4.10	168.56	0.00	0.04	1.45	0		<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
665	56.18	LAATNALLNSLEFTK	2	166.97	1.68	215.56	0.30	0.01	1.29	0.00	iTRAQ (Nterm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
666	15.87	LAATNALLNSLEFTK	3	57.94	2.89	89.64	1.96	0.05	1.55	0.03	iTRAQ (Nterm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
											Deamidation						

The information reported in the Peptide List includes scan number (spec.), identification score (score), peptide sequence (sequence), charge state (z), peak intensity (m/z 114, m/z 115, m/z 116, m/z 117), peptide ratios (Ra1, Ra2, and Ra3), labeling mode (Mod), and filtration information (L, S, U, R1, R2, R3). The last six attributes, L, S, U, R1, R2, and R3, indicate whether the peptide satisfies the corresponding filtration condition.

L: The box is checked if the peptide has been labeled by iTRAQ reagent.

- ❖ **S:** The box is checked if the peptide is considered as confidently identified and included for calculation of protein ratio (i.e., the peptide has identification over the score threshold set in the “Configure” box).
- ❖ **U:** The box is checked if the peptide is a non-degenerate (unique) peptide.
- ❖ **R1:** The box is checked if Ra1 is pass all the condition and use for calculate the protein ratio 1.
- ❖ **R2:** The box is checked if Ra2 is pass all the condition and use for calculate the protein ratio 2.
- ❖ **R3:** The box is checked if Ra3 is pass all the condition and use for calculate the protein ratio 3.

(The definitions of Ra1, Ra2, and Ra3 are similar to those in the Protein List.)

1.3 Data Chart

By clicking the scan no. (i.e., “spec.” in the Peptide List) of a peptide, the system will show its corresponding mass spectrum charts. Multi-Q provides the following four types of mass spectrum charts, and we show an example of “Raw Data” below.

- ❖ **Raw Data:** This chart shows original data in the mzXML file.
- ❖ **Smoothed Data:** This chart shows smoothed data.
- ❖ **Background:** This chart shows background information calculated by Multi-Q Web

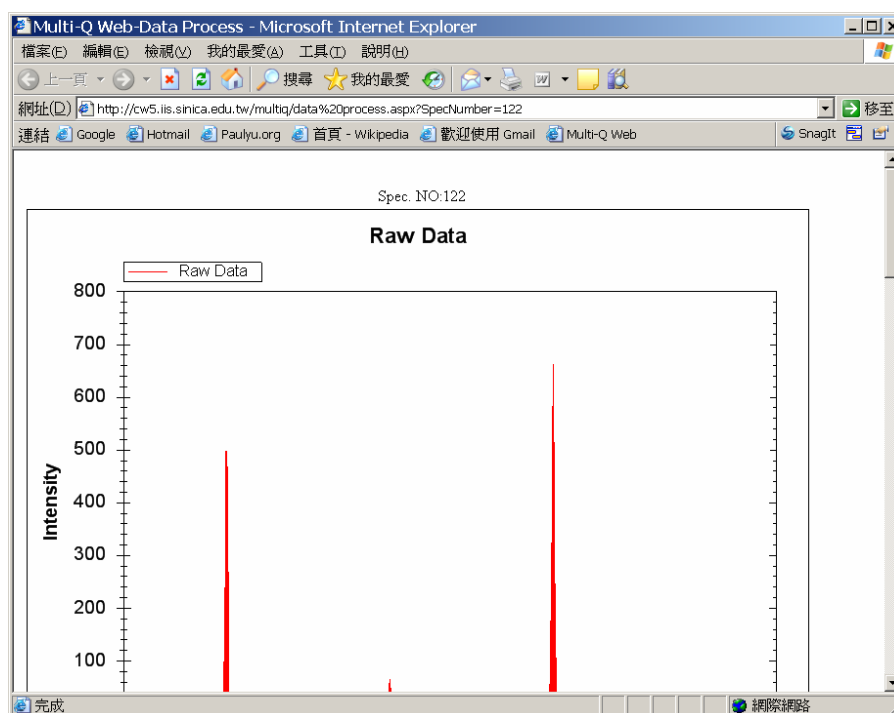
Server version.

- ❖ **Isotope Impurity Correction:** This chart shows the calculated intensities after isotope impurity correction with Software Correction Factors.

	No. of peptides with ratio not available	No. of peptides with ratio 0	No. of peptides with rational-value ratio	No. of peptides with infinity ratio	Calculational No.
R1	0(0/11)	3(3/11)	8(8/11)	0(0/11)	1
R2	0(0/11)	0(0/11)	11(11/11)	0(0/11)	3
R3	0(0/11)	1(1/11)	10(10/11)	0(0/11)	3

Peptide List : 11

spec.	score	sequence	z	m/z 114	m/z 115	m/z 116	m/z 117	R1	R2	R3	Mod	L	S	U	R1	R2	R3
50	5.7	VAAGLQIK	2	326.05	0.00	406.13	8.73	0	1.25	0.03	iTRAQ (Nterm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
122	27.72	VAALQNLVK	2	498.68	15.78	659.87	6.29	0.03	1.32	0.01	iTRAQ (Nterm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
550	45.4	LAATNALLNSLEFTK	2	115.92	4.10	168.56	0.00	0.04	1.45	0		<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
665	56.18	LAATNALLNSLEFTK	2	166.97	1.68	215.56	0.30	0.01	1.29	0.00	iTRAQ (Nterm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



1.4 Export Result

Click the “Save CSV format report” to save quantitation result in CSV format.

Output composition

Save CSV format report

Statistics of experiment results

Ratio 1(R1)	Ratio 2(R2)	Ratio 3(R3)
m/z 115	m/z 116	m/z 117
m/z 114	m/z 114	m/z 114

	No. of proteins with ratio 0	No. of proteins with rational-value ratios	Normalization Factor
R1	36 (36/115)	79 (79/115)	-0.001
R2	30 (30/115)	85 (85/115)	1.185
R3	32 (32/115)	83 (83/115)	0.013
	No. of proteins with std not available	No. of proteins with std 0	No. of proteins with rational-value std
Std 1	34 (34/115)	36 (36/115)	45 (45/115)
Std 2	27 (27/115)	30 (30/115)	58 (58/115)
Std 3	31 (31/115)	32 (32/115)	52 (52/115)

	A	B	C	D	E	F
1	Multi-Q Quantitation Result					
2						
3	Configuration Section Begin					
4	Config Name Config					
5	Smoothing	0				
6	Data type	0				
7	Peptide Score	0				
8	Nondegenerate	TRUE				
9	Weighted confidence	TRUE				
10	Dynamic Range	0				
11	Dynamic Range	0				
12						
13	Isotope Impurity Correction Table					
14	Reagent	% of -2	% of -1	% of +1	% of +2	
15	iTRAQ Reagent	-	-	0.059	0.002	
16	iTRAQ Reagent	-	0.02	0.056	0.001	
17	iTRAQ Reagent	0	0.03	0.045	-	
18	iTRAQ Reagent	0.001	0.04	-	-	
19						
20						
21	Ratio Table					
22		Ratio 1(R1)	Ratio 2(R2)	Ratio 3(R3)		
23	Numerator	m/z 115	m/z 116	m/z 117		
24	Denominator	m/z 114	m/z 114	m/z 114		
25						
26	Configuration Section End					
27						
28	"NonD" stands for is Nondegenerate peptide					
29						
30	AccNo	Score	Mass	Ratio1	Ratio2	Ratio3
31	IMB1_HUI	158	97108	0.031634	1.291965	0.01918
32	spec.	score	sequence	z	m/z 114	m/z 115
33	50	5.7	VAAGLQI	2	326.0486	0
34	100	0.000	VAAGLQI	0	400.0045	15.0000

2. Customized Output Reports

Users can customize their output reports by clicking “Output composition” at the top of the default output page. Then an output format setting panel will pop up for users to determine report parameters for composing their report.

Output composition

[Save CSV format report](#)

Statistics of experiment results

Ratio 1(R1)	Ratio 2(R2)	Ratio 3(R3)
m/z 115	m/z 116	m/z 117
m/z 114	m/z 114	m/z 114

	No. of proteins with ratio 0	No. of proteins with rational-value ratios	Normalization Factor
R1	36 (36/115)	79 (79/115)	-0.001
R2	30 (30/115)	85 (85/115)	1.185
R3	32 (32/115)	83 (83/115)	0.013
	No. of proteins with std not available	No. of proteins with std 0	No. of proteins with rational-value std
Std 1	34 (34/115)	36 (36/115)	45 (45/115)
Std 2	27 (27/115)	30 (30/115)	58 (58/115)
Std 3	31 (31/115)	32 (32/115)	52 (52/115)

The parameters are introduced below.

- ❖ **Protein table field:** Users can assign protein information fields in output results.
- ❖ **Peptide table field:** Users can assign peptide information fields in output results.
- ❖ **Color Setting:** Users can assign preferred colors for various output information.
- ❖ **HighLight Setting:** Users can also assign preferred colors for some specific fields that satisfy given conditions.

Fields of protein summary	
Not to be shown in the summary	To be shown in the summary
	AccNo Score Mass Ratio1 Ratio2 Ratio3 STD1 STD2 STD3 Description

Fields of peptide summary	
Not to be shown in the summary	To be shown in the summary
	spec. score sequence z m/z 114 m/z 115 m/z 116 m/z 117 Ratio 1 Ratio 2 Ratio 3 Mod Is Label Is Score Is Unique R1 R2 R3

Color setting	
Header of protein summary	Blue
Row of protein summary	LightSteelBlue
Header of peptide summary	Lavender
Row of peptide summary	OldLace

HighLight Setting		
<input type="checkbox"/> Protein Score >		AliceBlue
<input type="checkbox"/> Protein Score <		AliceBlue
<input type="checkbox"/> Protein Ratio >		AliceBlue
<input type="checkbox"/> Protein Ratio <		AliceBlue
<input type="checkbox"/> Protein STD >		AliceBlue
<input type="checkbox"/> Protein STD <		AliceBlue
<input type="checkbox"/> Peptide Ratio >		AliceBlue
<input type="checkbox"/> Peptide Ratio <		AliceBlue
<input type="checkbox"/> Score > peptide score	True	AliceBlue
<input type="checkbox"/> Is Label	True	AliceBlue
<input type="checkbox"/> Is nondegenerate peptide	True	AliceBlue
<input type="checkbox"/> Is R1 available	True	AliceBlue
<input type="checkbox"/> Is R2 available	True	AliceBlue
<input type="checkbox"/> Is R3 available	True	AliceBlue

Then users just click the “Generate” button to generate a result webpage. An example of customized output report is shown below.

Multi-Q Quantitation Result	
Date	2007/1/29
Time	下午 03:08
Search Name	F004675.csv
Config	
Smoothing and background subtraction	Not Apply
Data type	Sample
Peptide Score >	0
Nondegenerate Peptide	Use nondegenerate Peptide
Weighted calculation	Apply
Dynamic Range	
Low Intensity	0
High Intensity	0

Isotope Impurity Correction Table				
Reagent	% of -2	% of -1	% of +1	% of +2
iTRAQ Reagent 114	-	-	0.059	0.002
iTRAQ Reagent 115	-	0.02	0.056	0.001
iTRAQ Reagent 116	0	0.03	0.045	-
iTRAQ Reagent 117	0.001	0.04	-	-

Ratio			
	Ratio 1(R1)	Ratio 2(R2)	Ratio 3(R3)
Numerator	m/z 115	m/z 116	m/z 117
Denominator	m/z 114	m/z 114	m/z 114

AccNo	Score	Mass	Ratio	Ratio2	Ratio3	STD1	STD2	STD3	Description							
IMB1_HUMAN158	971080.03	1.29	0.02	N/A	0.05	0.03			(Q14974) Importin beta-1 subunit (Karyopherin beta-1 subunit) (Nuclear factor P97) (Importin 90)							
spec. score sequence			z m/z 114	m/z 115	m/z 116	m/z 117	Ratio 1	Ratio 2	Ratio 3	Mod	Is Label	Is Score	Is Unique	R1	R2	R3
50	5.7	VAAGLQIK	2326.04860		406.13498	726358	0	1.25	0.03	iTRAQ (Nterm)	True	True	True	False	True	True
122	27.72	VAALQNLVK	2498.684515	7753	659.86766	290183	0.03	1.32	0.01	iTRAQ (Nterm)	True	True	True	True	True	True
550	45.4	LAATNALLNSLEFTK	2115.91794	104576	168.56190		0.04	1.45	0		False	True	False	False	False	False
665	56.18	LAATNALLNSLEFTK	2166.96641	681888	215.56	0.29812010	0.01	1.29	0.00	iTRAQ (Nterm)	True	True	False	False	False	False
666	15.87	LAATNALLNSLEFTK	357.9422	2.890135	89.643751	9.63141	0.05	1.55	0.03	iTRAQ (Nterm)	True	True	False	False	False	False
667	32.54	LAATNALLNSLEFTK	2103.97091	454977	113.59512	886765	0.01	1.09	0.03	Deamidation (NQ); iTRAQ (Nterm)	True	True	False	False	False	False
697	29.89	AAVENLPTFLVELSR	265.980930	953239671	703762	772377	0.01	1.09	0.04	iTRAQ (Nterm)	True	True	False	False	False	False
698	41.98	AAVENLPTFLVELSR	329.970271	486627	24.7414	2.88515	0.05	0.83	0.10	iTRAQ (Nterm)	True	True	False	False	False	False
757	52.85	LAATNALLNSLEFTK	2121.002	0	198.72141	0.57639	0	1.64	0.01	iTRAQ (Nterm); iTRAQ (K)	True	True	False	False	False	False
758	7.07	LAATNALLNSLEFTK	375.966281	686031	127.62343	2.55259	0.02	1.68	0.04	iTRAQ (Nterm); iTRAQ (K)	True	True	False	False	False	False
897	35.2	YLEVVLNITLQASQAQVQDK	317.012670		20.959161	0.57472	0	1.23	0.06	iTRAQ (Nterm)	True	True	True	False	True	True

AccNo	Score	Mass	Ratio	Ratio2	Ratio3	STD1	STD2	STD3	Description							
K220_HUMAN49	658300.01	1.24	0.02	0.00	0.08	0.01			(Q01546) Keratin, type II cytoskeletal 20a (Cytokeratin 2P) (K2P) (CK 2P)							
spec. score sequence			z m/z 114	m/z 115	m/z 116	m/z 117	Ratio 1	Ratio 2	Ratio 3	Mod	Is Label	Is Score	Is Unique	R1	R2	R3
130	1.2	FASFIDK	2555.12980		691.09693	9.0713	0	1.24	0.01	iTRAQ (Nterm); iTRAQ (K)	True	True	False	False	False	False
216	19.91	FASFIDKVR	3135.97741	128131	161.51563	7.30668	0.01	1.19	0.03	iTRAQ (Nterm)	True	True	False	False	False	False
266	7.2	SEAEALYQTK	236.992390	380476847	870890	84542940	0.01	1.29	0.02	Deamidation (NQ); iTRAQ (Nterm)	True	True	True	True	True	True
360	19.19	FASFIDKVR	3455.88445	781593	610.42358	5.25163	0.01	1.34	0.02	iTRAQ (Nterm); iTRAQ (K)	True	True	False	False	False	False
611	23.17	YTLNILEEIGGGQK	2136.98650	6777865174	5.6233	1.44018	0.00	1.27	0.02	Deamidation (NQ); iTRAQ (Nterm)	True	True	False	False	False	False
811	35.69	LDSEDKDKGKPLK	498.011960		93.806270	7.7931540	0.96	0.01		iTRAQ (Nterm); iTRAQ (K)	True	True	False	False	False	False
857	9.68	DANAKLQDLQALQKAK	4381.94012	994239	448.91663	7.95758	0.01	1.18	0.01	2 Deamidation (NQ); 2 iTRAQ (K)	True	True	True	True	True	True
959	10.22	NTKSEIMELNRMIQRLRAEIVK	5228.07190		304.53335	2.99597	0	1.34	0.02	Deamidation (NQ); 2 Oxidation (M); iTRAQ (K)	True	True	True	False	True	True

Figure: An example of customized output report