

### **PEAKS** 5 Studio User's Manual



BIOINFORMATICS SOLUTIONS INC

### PEAKS Studio 5 User's Manual

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Please contact BSI for questions or suggestions for improvement.

1.	Introd	luction to PEAKS 5	6
		Main Features	
	1.2	New Features in PEAKS 5	. 6
	1.3	Workflow	.7
	1.4	Guidelines for Using this Manual	.7
	1.5	Scope	.7
	1.6	Service and Support	.7
2.		g Started with PEAKS 5	
		Package Contents	
		System Requirements	
		Installation for Windows Users	
	2.4	Registering PEAKS	10
		Set up PEAKS Preferences	
		Set up PEAKS Configuration	
3.		Walkthrough1	
	•	Create a Sample Database	
		Create a Project	
		Perform Data Refinement	
		Run <i>De novo</i> Sequencing	-
		Run Protein Identification	
		Run PTM Finder	
		Run an inChorus Search	
		Perform a SPIDER Search	
4.		data	
		Data Format	
		Data Conversion	
		Thermo Data	
		Agilent Data	
		Bruker Data	
		Shimadzu Data	
		Applied Biosystems Data	
		Varian Data	
		Waters/Micromass (MassLynx) Data	
		ABI 4700 or 4800 Data	
	4.3	Load a New File	
		Create a New Project	
		Open a Project	
		Changing the Location of Saved Projects	
		Orienting Yourself	
		Project View Panel	
		Properties Panel	
		Raw Spectrum View	
5.	Data r	efinement	
- •		Run Data Refine	

	5.2 Data Refinement Parameters	
	Merging Scans	
	Precursor Charge Correction	
	Filtering MS/MS Scans	
	Preprocessing MS/MS Scans	
	5.3 Data Preprocessing Results	
6.	De novo Sequencing	
	6.1 Setting up Auto <i>De novo</i> Sequencing Parameters	
	Mass Options	
	Enzyme Options	
	PTM Options	
	General Options	
	6.2 De novo Sequencing Results	
	Peptide Candidates Frame	
	Ion Table Frame	
	Spectrum View Frame	
	Spectrum Alignment Frame	
	Survey Scan	
	Error Map	
7.	Database Search	
	7.1 Setting up Protein Identification Parameters	
	Mass Options	
	Enzyme Options	
	General Options	
	PTM Options	
	Database Options	
	Advanced Options	
	7.2 Protein Identification Results	
	Peptide View	
	Protein View	
	Chart View	
8.	SPIDER Search	63
	8.1 Setting up SPIDER Parameters	
	Query Options	
	General Options	
	PTM Options	
	Filter Options	
	De novo Options	
	Database Options	
	8.2 SPIDER Results View	
9.	PTM Finder	68
	9.1 Setting up PTM Finder Parameters	
	9.2 PTM Finder Results View	
10.	inChorus Meta Search	
	10.1 Setting up inChorus Parameters	
	Importing Existing Results	

	10.2 inChorus Results View	
	De novo, Peptide and Protein Views	.72
	Chart View	.72
11.	Filtering Your Results	.73
	11.1 Setting Filter Parameters	.73
	Possible Filters/ Selected Filters/ Edit Filter	. 74
	Filter Options	.75
	Parameter Options	
12.	Complex Analysis	.78
	12.1 Creating a project for complex system	
	12.2 Integrating data analysis	
13.	Exporting Data/Reports and Printing	
	13.1 Export Data in .mzxml or .mgf	
	13.2 Export Peptide Results in PepXML Format	
	13.3 Export Results in Excel Format	
	13.4 Print Tables and Graphs for Publication	
	De novo Image Files	
	Protein ID Image Files	
	inChorus Image Files	. 86
	Compare Image Files	
14.	Advanced Configuration and Environment Preferences	.88
	14.1 PEAKS Environment Preferences	
	General Preferences	. 89
	Instrument Preferences	. 91
	Search Engine Preferences	. 93
	Ion Editor Preferences	
	14.2 PEAKS Configuration	
	Enzyme Configuration	
	PTM Configuration	
	Database Configuration	
	Instrument Configuration	
	Parameter Configuration	
15.	PEAKS Quantification 1	106
	15.1 Setting up PEAKS Q Parameters	
	15.2 3D View	
	15.3 iTRAQ Walkthrough	
	1) Creating a Project	
	2) Running data refinement	
	3) Running PEAKS Search	
	4) Running Quantification	
	15.4 SILAC Walkthrough	
	1) Creating a project	
	2) Running Protein Identification	
	<ul> <li>3) Running Protein Identification</li></ul>	
	4) Running Quantification	
	15.3 Label Free Quantification (Available in future)	120

16.	References	
	De novo	
	SPIDER	
	Quantification	
18.	Appendix	
	18.1 Terminology and Abbreviations Glossary	
	18.2 Toolbars	
	Main Window Toolbar	
	Project View	
	Main Processing Window Toolbar	
	18.3 Mass Calculator	
	Advanced Options	
19	About Bioinformatics Solutions Inc.	
20	PEAKS Software License	



#### 1. Introduction to PEAKS 5

#### **1.1 Main Features**

PEAKS is an innovative software system designed to derive amino acid sequences and identify proteins using tandem mass spectrometry data from all major mass spectrometry vendors. PEAKS incorporates *de novo* sequencing results into the database searching process for peptide/protein identification. It does this by generating sequence tags which are used in conjunction with fragment ion mass matching to speed up the search, remove false positive matches, and find peptides with interesting sequence variations or modifications that would prevent them from being otherwise identified. Our meta protein search tool, inChorus allows users to use multiple search engines (PEAKS, Sequest, Mascot, X!Tandem and OMSSA) to expand sequence coverage and increase confidence. Another tool, SPIDER, is used to reconstruct the correct sequence using the *de novo* sequence and a homologous peptide.

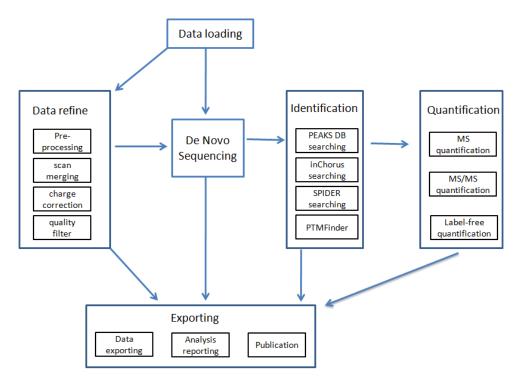
#### 1.2 New Features in PEAKS 5

We have many new features in PEAKS 5 which will be explained throughout this manual. PEAKS 5 is now capable of handling very large data sets. Our protein identification is more sensitive and generates less false positives.

PEAKS 5 also has improved identification of PTMs with our new PTM finder. *BSI*, makers of PEAKS, has also created a quantification module which will allow users to automatically quantify proteins from experiments using both label and label-free techniques. Results generated from PEAKS Q have high accuracy and can be performed over a wide dynamic range. Please note that the label-free quantification protocol is not included in this first release.

PEAKS 5 uses project based data management which allows users to process simultaneous runs and easily compare/contrast samples within one project.

#### 1.3 Workflow



#### 1.4 Guidelines for Using this Manual

This user's manual is intended to help you get started with PEAKS 5. It will describe its functionalities, show how to customize PEAKS to your applications, provide a task based reference, and troubleshooting. We recommend reading the walkthrough in Chapter 3, using the sample data provided.

#### 1.5 Scope

PEAKS users are assumed to be familiar with computer usage and the operating system environment. As such, it is beyond the scope of this manual to instruct the user on the use of windows, dialogue boxes, menus, file storage etc. Please refer to the operating system's manual or computer help books for such information. Similarly, PEAKS users are expected to be familiar with mass spectrometry, standard operating practices and data.

#### 1.6 Service and Support

In addition to reading this manual, we recommend that you take a look at our training videos that explain the main features of PEAKS in detail

(http://bioinfor.com/products/peaks/support/tutorials.php). Please send technical questions to support@bioinfor.com.

We also encourage our users to provide us with any suggestions or comments as we are always improving PEAKS to meet the needs of the scientific community (http://www.bioinformaticssolutions.com/corporate/contactform.php).

# Chapter

#### 2. Getting Started with PEAKS 5

This section of the manual will guide you through the installation and configuration of PEAKS 5.

#### 2.1 Package Contents

The PEAKS 5 package contains:

- This manual (hardcopy and/or electronic version)
- PEAKS 5 software

#### 2.2 System Requirements

PEAKS 5 will run on most platforms with the following requirements:

- Processor: Equivalent or superior processing power to a Pentium III at 800 MHz.
- Memory: 1 GB minimum, (1.5GB is recommended) for PEAKS Studio / PEAKS Client. 500MB is recommended for PEAKS Viewer.
- Operating System: PEAKS runs on Windows Vista, XP and Linux.

#### Adjusting the Amount of Memory that PEAKS Uses

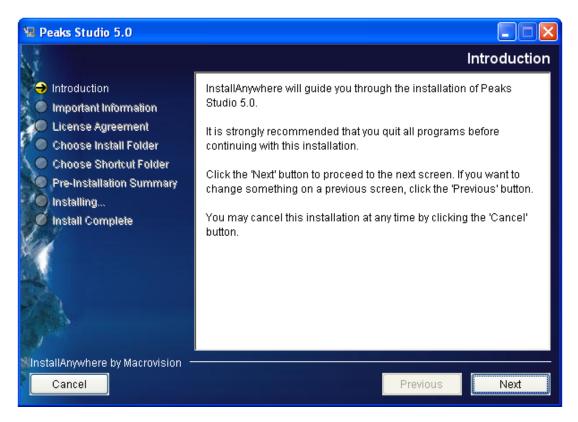
The PEAKS Studio directory (e.g., C:/PEAKS Studio 5.0) contains a file called StartPEAKSStudio.lax which contains a line which looks like *lax.nl.java.option.java.heap.size.max=1200m*. The 1200m tells the Java VM (which runs PEAKS) to run with 1200MB of memory. With some trial and error (the Java VM will fail to run if the setting is too high) you can determine the highest optimal value for your own computer.

#### 2.3 Installation for Windows Users

Note: If you already have an older version of PEAKS installed on your system, please uninstall it before proceeding.

- 1. Close all programs that are currently running.
- 2. Insert the PEAKS 5 disc into the CD-ROM drive. If loading PEAKS via the download link, skip to step 4, after downloading and running the file.

- 3. Auto-run should automatically load the installation software. If it does not, find the CD-ROM drive and open it to access the disc. Click on the PEAKS\_Studio\_Installation.exe.
- 4. A menu screen will appear. Select the top item "PEAKS Installer." The installation utility will begin the install. Wait while it does so. When the "PEAKS 5" installation dialogue appears, click the "Next" button.



- 5. Basic system requirements will be presented. Please note that while the minimum requirement is 1 G of RAM, the preferred configuration is 1.5 G of RAM. "Click Next".
- 6. Read the license agreement. If you agree with it, change the radio button at the bottom to select "I accept the terms of the License Agreement" and click "Next".
- Choose the folder/directory in which you would like to install PEAKS. The default location is simply "C:\PEAKS 5. To change this location press the "Choose..." button to browse your system and make a selection, or type a folder name in the textbox. Click "Next".
- 8. Choose where you would like to place icons for PEAKS 5. The default will put these icons in the programs section of your start menu. A common user preference is on the desktop. Click "Next".

- 9. Review the choices you have made. You can click "Previous" if you would like to make any changes or click "Next" if those choices are correct.
- 10. PEAKS 5 will now install on your system. You may cancel at any time by pressing the "Cancel" button in the lower left corner.
- 11. When the installation is complete, click "Done". The PEAKS 5 menu screen should still be open. You may view movies and materials from here. To access this menu at a future date, simply insert the disc in your CD-ROM drive.

#### 2.4 Registering PEAKS

The first time PEAKS is run, you will be told that the product is not registered. Press the "OK" button and a dialogue will appear. Follow the onscreen instructions depending on your requirements. For users entitled to a perpetual license, select the "Request License file" and click "Next". In the field that appears, enter the registration key that came with the product – whether it is a key for the full version. You must also enter your name, the name of your organization. An automated servant from BSI will generate the license file (license.lcs) and email it to the provided email account from the License Wizard. Save the license file to a local directory and then continue with the License Wizard to import the license file into the PEAKS folder. A dialogue box will then read "Registration Successful".

🔺 License Wizard 🛛 🛛 🔀
License Wizard
Please select one of the following tasks:
Request a 30 days evaluation license
<< Back Next >> Cancel Help

If you are not connected to the internet, onscreen instructions will offer assistance for offline registration.

Re-registering PEAKS may be necessary if your license has expired or if you wish to update the license. You will need to obtain a new registration key from BSI. Once you have obtained this new key, select "About PEAKS Studio" from the Help menu. The "About BSI PEAKS Studio"

dialogue box will appear. Press the "License Wizard" button to continue. Follow the on-screen instructions.

bout BSI PEAK	S Studio					
	PEAKS Studio 5.0 build 200	PEAKS Studio 5.0 build 20081107				
	Copyright © 2000-2009 Bi	oinformatics Solutions Inc. All rights reserve	ed.			
	C:\Peaks Studio 5.0\thirdpartylicense.txt					
Carle I	License to	A Lab Inc.				
Carlos I	License key	PS##########				
Saul -	Register email	aperson@alabinc.com				
a the second	License start / expire	2009-02-01 / Never Expires				
1402	SPS expire	N/A				
114	Thread #	1				
	Module	License status				
120	PEAKS Protein ID	Never Expires				
	PEAKS Platform	Never Expires				
A STATE	PEAKS De novo	Never Expires				
Contra Contra	SPIDER	Never Expires				
and the second	PEAKS Q	Never Expires				
	View end user license agr	eement				
And Street	and international treaties.	rogram is protected by copyright law Unauthorized reproduction or n, or any portion of it, may result in	ОК			
BSi		enalties, and will be prosecuted to the	License Wizard			
Concella			Tech support			

#### 2.5 Set up PEAKS Preferences

Before running your data, you must set up search engine preferences. For an explanation on how to do this, see page 93.

It is possible to run your data through PEAKS without changing the other preferences as they have default settings. For more information on changing these default settings see page 81.

#### 2.6 Set up PEAKS Configuration

Before running your data, you must configure your databases. For instructions on how to do this, see page 99.

It is possible to run your data through PEAKS without configuring any other parameters as they have default settings. For more information on changing these default settings see page 97.



#### 3. Quick Walkthrough

This section of the manual will walk you through most of the basic functionality of PEAKS 5. After completing this section you will see how easy it is to load, view a data file, perform data refinement, perform *de novo* sequencing, and database search protein identification.

Please note that version upgrades of PEAKS as well as upgrades to the databases may result in small changes to the results screenshots in this chapter.

#### 3.1 Create a Sample Database

Before running the walkthrough data, you need to set up a database. So that this can be a quick process, we have provided you with a sample fasta database called "SampleDB.fasta" in your PEAKS program folder (C:\PEAKS Studio 5.0\Data).

Click on the configuration toolbar icon  $\overset{\sim}{\sim}$  or select "Configuration" from the "Tools" menu. Select "Database" from the left hand side of the window. Under "Database Details" enter the following information:

Database Details				
FASTA format database:		UniProtKB/Swiss-Prot	*	
Basic Options	5			
Database n	iame:	Sample DB		Download Database
Path: C:\Peaks Stu		dio 5.0\Data\SampleDB.fasta		Browse
🗌 EST da	tabase			

You do not need to change any of the other information listed. Click the "Add/Update" button and then click "OK".

#### 3.2 Create a Project

This will be a rather simple project as it will only contain one sample, however the same process will be used for projects with multiple samples and files. Click on the "Create new project" button for select "New Project" from the "File" menu. The following window will appear:

📐 New Project		×	
Steps	Project Properties		Enter a
1. Project Properties	Project Name:	Orbisample	name for your
2	Project Location:	C:\Peaks Studio 5.0\.\derbyServer\serverDB Browse	project.
	Project Folder:	:\Peaks Studio 5.0\.\derbyServer\serverDB\Orbisample	Click the
	Notes/Description:		"Next"
			button.
	Type and organization	of project:	
	<ul> <li>Basic Project</li> </ul>		
	<ul> <li>Several non-labella</li> </ul>	ed samples for comparison (each sample can be fractionated)	
		<< Back Next >> Cancel Help	

🔼 New Project					By default the
Steps 	Sample Properties	Sample 1		~	first sample will be named "Sample 1".
2. Sample Properties 3	Select Files: Name Siz		Date Modified	Туре	Click the "Add a file for this sample" button. Select the "OrbiSample.
	Add a file for this sample Sample Notes/Description:	Remo	ve file from list	Clear list	mzxml" file from your PEAKS program folder.
		Add another	sample Rer	move current sample	(For example "C:\PEAKS Studio
		<< Back	Next >> Car	ncel Help	5.0\Data")

📐 New Project	New Project				×	
Steps	Sample Properties					
1. Project Properties	Give this sample a name:	Sample 1				*
2. Sample Properties	Select Files:					
3	Name	Size		Date Modified	Туре	
	ata\OrbiSample.mzXML		370 KB	01/21/2009 17:06:05 PM		mz×ml

Click "Next".

🔼 New Project	×				
Steps	Instrument Details				
1. Project Properties	Instruments(s) used to acquire the data:				
2. Sample Properties					
3. Instrument Details	_General Instrument Details: FT-trap				
4	FT-trap   FT-trap   FT-trap (ecd-cid)   FT-trap (etd)   FT-trap (etd)   FT-trap (pqd)   FTMS   FTMS (ecd)   FTMS (ecd-cid)   FTMS (etd)				
	<< Back Next >> Cancel Help				

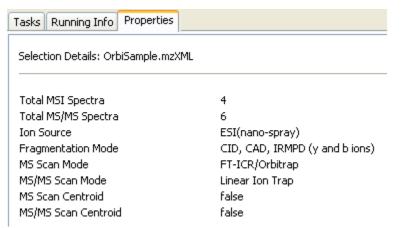
We will leave the instrument vendor as "General" and select the instrument to be "FT-trap". Click "Next". You should now see the file in the "Project View" panel:

📐 Project View 🛓 🔟 C:/Peaks Studio 5.0/./derbyServer/serverDB/New Project 1 🖮 🛴 Sample 1 OrbiSample.mzXML

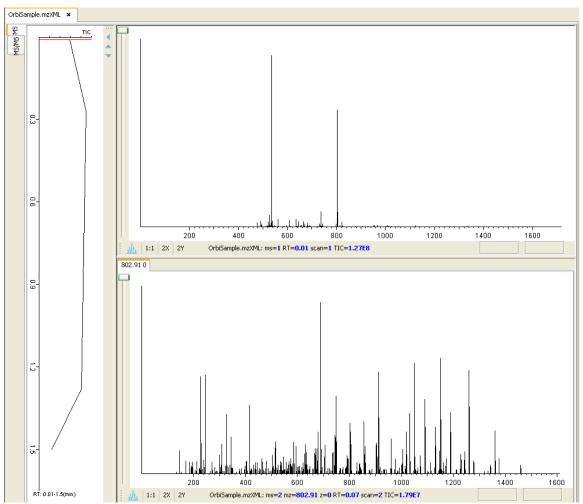
The "Project View" panel which is shown in the upper left hand corner displays the organization of a particular project. Use the '+' and '-' boxes to expand and collapse the project in order to access the

data file in the "Project View". Make sure that you select this data file when choosing data to be analyzed.

PEAKS reads and tracks information about the experiment and data for use in the analysis and for future reference. Once the data file has loaded, click on the "Properties" tab in the bottom left hand corner:



#### You should see the following in the "Main Processing Window":



The information that is displayed by default pertains to the precursor scan. To the left of the window is the "Total Ion Current" (TIC). The graph in the upper right corner displays a survey scan with its corresponding tandem scans below. Click on the MS/MS tab to see the graphs that were generated from the tandem scan. For more information on the functions and tools found in these windows, see page 40.

#### 3.3 Perform Data Refinement

1) Click the Data Refine toolbar icon  $\propto$ Or

Select "Data Refine" from the "Tools" menu.

Data Refine	×
ools	Data Refine
Data Refine De novo PEAKS Search	Merge Options         Merge scans of the same peptide:       () yes       () no         Retention time window: (for raw files only)       min.         m/z tolerance:       Da
SPIDER Search PTM Finder Quantification	Charge Options Correct precursor charges:       Orrect precursor charges: <ul> <li>yes</li> <li>no</li> </ul> Minimum charge:         1         Maximum charge:         4
	Filter Options         Filter MS/MS scans: <ul> <li>Precursor mass between</li> <li>and</li> <li>Da</li> <li>Retention time between</li> <li>and</li> <li>min</li> <li>Quality value greater than</li> <li>0.65</li> </ul>
	Preprocess Options Preprocess MS/MS scans: O no, already done ④ yes O no
	OK Cancel Help

2) Enter the settings as shown:

For more details on setting up the parameters for data refinement refer to page 43.

Here we will use a quality filter to remove data with a quality value lower than 0.65. As all of the data in this data set is of good quality data, we will not remove any data using this filter.

Tasks Running Ir	nfo Properties	
Selection Details:	OrbiSample.mz>	(ML
Total MSI Spectri Total MS/MS Spe- Ion Source Fragmentation M MS Scan Mode MS/MS Scan Cantroic MS/MS Scan Ceni Group MS/MS Sca Charge Options Filter Quality Process	ctra ode le d troid	4 6 ESI(nano-spray) CID, CAD, IRMPD (y and b ions) FT-ICR/Orbitrap Linear Ion Trap false false false 6 [1 - 4] >0.65 true
<		

After running data refine, there will be new information listed in the "Properties" file.

#### 3.4 Run De novo Sequencing

1) Click the *De novo* sequencing toolbar icon *a Or* 

Select "De novo" from the "Tools" menu.

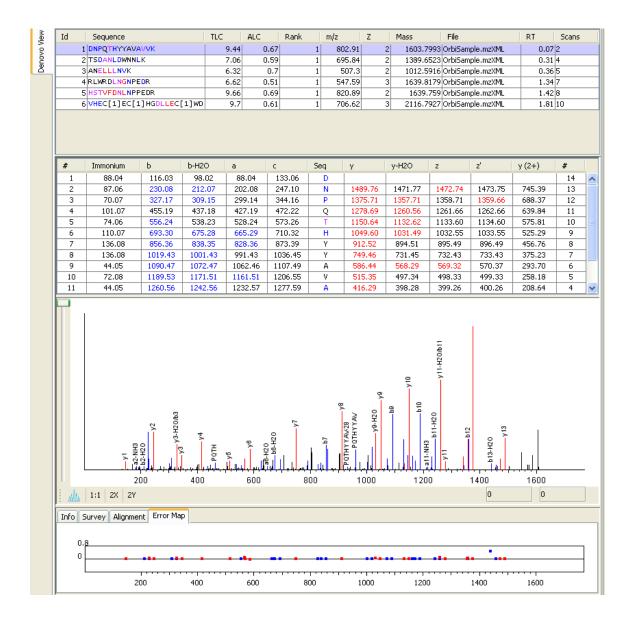
2) Enter the settings as shown:

Data Refine De novo	Mass Options Parent Mass Error To							
	Parent Mass Error To							
De novo		lerance:	0.1					Da 💌
	Fragment Mass Error	Tolerance:	0.8					Da
PEAKS Search	Enzyme Options							
5	Enzyme:	Tr	ypsin			×	New	Enzyme
5PIDER Search	Digest Rule:	RK	,		{P}		Deleta	Environ
PTM Finder	Digest Rule:	KN	·		(P)		Delete	e Enzyme
	Find peptides that	t satisfythe abo	ive rule at both en	nds			Ad	vance
Quantification								
	PTM Options							
	Sector Sector Sector							
	and the second se	Mono mass	Residue site		Add Fixed =>	Fixed Modification		
			[M]@C	1	<= Remove	Todoacetic acid der	ivative	
	Homoserine lac4 Hydroxylation 15	8.0034 5.9949	[M]@C [PKDNRY]	_				
		12	[K], [X]@N	/				
		)5.0215	[K], [X]@N		Add Variable =>	Variable Modification		
	Iodoacetic acid 58		[C]		<= Remove			
	and the second sec		[K]	- U	<= Remove			
	Show unimod	[	New PTM			Max variable PTM per pe	ptide:	3 🗘
		_						
	General Options							
	Preprocess this	data 'on the fly'	'(deconvolute filt	ter nois	e centroid)	Report up to (	# nentides	s): 1 🛟
		add on the hy	(4000) 110		, controloy	insport up to (	" populati	

Note that we are not going to preprocess this data "on the fly" as we have already preprocessed the data during the data refinement stage. We will also choose to report only one peptide per spectrum for simplicity's sake.

You can save the parameters that you used for future reference by clicking on the "Save Parameter" button. For more information on setting up *de novo* parameters see page 44. Click "OK" to commence analysis. For this sample it takes just over a minute. The PEAKS auto *de novo* algorithm derives sequence candidates for each of the six spectra in our example data file. Take a look at spectra ID 1. Notice that the number in square brackets refers to the modification which in this case is iodoacetic acid derivative.

After *de novo* sequencing is complete, the following will appear in the "Main Processing Window":



At the top of the screen you will see the peptide candidates in the "Peptide Candidates Frame". The peptide candidates are sorted by "ID". Right next to the proposed sequence, the auto *de novo* "Total Local Confidence" (TLC) and "Average Local Confidence" (ALC) confidence scores are shown. You will also see the m/z ratio, mass, retention time etc. listed for each peptide sequence. For information including color coding, see page 47.

Below the "Peptide Candidates Frame" is the "Ion Table Frame". Each amino acid is colorcoded by confidence level (see page 47) with the masses for matched a, b and c ions listed in blue and for the matched x, y and z ions listed in red.

Below the "Ion Table Frame" is the "Spectrum View Frame". This frame is useful for seeing the strength of the ms/ms peaks that PEAKS 5 has set as ions. Here the alignment of the assigned b (blue) and y (red) ions with the entire spectrum corresponding to the selected peptide can be observed. For more information on the "Spectrum View Frame", see page 49.

At the bottom of the page is the "Error Map", which displays the confidence that is assigned to each ion. The most confident results lie on the centerline. For more information on the "Error Map", see page 50.

#### 3.5 Run Protein Identification

1) Click the PEAKS Search toolbar icon  $\boxed{Or}$ 

Select "PEAKS Search" from the "Tools" menu.

2) Enter the settings as shown:

PEAKS Search	
Tools	PEAKS Protein ID
. Data Refine	
. Data Kenne	Database Search Save Parameter orbisample
. De novo	Mass Options General Options
✓ PEAKS Search	Parent Mass Error Tolerance:       0.1       Da       Precursor Mass Search Type:       Preprocess this data 'on the fly'         Fragment Mass Error Tolerance:       0.8       Da       Image: Max Missed Cleavages;       1 Image: Max Missed Cleavages;
. SPIDER Search	Enzyme Options
. PTM Finder	Enzyme: Trypsin Vew Enzyme
. Quantification	Digest Rule: RK [P] Delete Enzyme
	Find peptides that satisfythe above rule at both ends
	PTM Options
	Name         Mono mass         Residue site         Add Fixed =>         Employee
	ICPL-heavy         111.0416         [K], [X]@N         ICPL-light         Iodoacetic acid derivative           ICPL-light         105.0215         [K], [X]@N         Image: Comparison of the second derivative         Image: Comparison of the second derivative
	Iodoacetic acid 58.0055 [C]
	Methyl ester 14.0156 [DE], [X]@C Add Variable => 🔁 Variable Modification
	Methylation         14.0156         [CKRHDENQ],           O18 label         2.0042         [STY], [X]@C
	Propionamide 71.0371 [C]
	Show unimod New PTM Max variable PTM per peptide: 3
	Database Options
	Select database Select Database: Sample DB ▼
	Paste fasta sequences     New Database     Edit Database
	Set Taxa
	Advanced Options
	PEAKS uses a hybrid search technique that requires some sequence tags to help in the search <ul> <li>I have already run de novo, don't run it again</li> </ul>
	O Run de novo using different parameters than the above orbisample
	O Run de novo using the same parameters as above (default)
	Validation - decoy search

Parameters can be saved and used for future reference by clicking on the "Save Parameter" button. For more information on setting up protein identification parameters see page 51.

Click "OK" to commence analysis.

After PEAKS Protein ID is completed, the click on the "*De novo*" view tab. Recall that PEAKS found *de novo* sequencing results for all six spectra, however only four spectra (ID 1, 3, 4, 5) had a corresponding proteins found in the database.

Id	Sequence	TLC	ALC	Rank	m/z	Z	Mass	File
	1 DNPQTHYYAVAVVK	9.44	0.67	1	802.91	2	1603.7993	OrbiSample.mzXML
	3 ANELLLNVK	6.32	0.7	1	507.3	2	1012.5916	OrbiSample.mzXML
	4 RLHC[1]C[1]LNGNPEDR	6.5	0.5	1	547.59	3	1639.7307	OrbiSample.mzXML
	5 HSTVFDNLNPPEDR	9.68	0.69	1	820.89	2	1639.759	OrbiSample.mzXML

Now click on the "Peptide View" tab. The following will appear in the "Main processing window":

🐻 Peptides			AKS(Score %)	RSD	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(pp
		AVVK	99.0						
			97.32						
Spectrum 3			97.32		507.3	2			:
🗄 🕘 Hit 5	HSTVFDNLPNF	EDR	99.0				1639.759	J	
Peptide Align Pr	eptide Details								
#	Immonium	Ь	Ь-Н2О	а	с	9	ieq y	y-H2	20
1	88.04	116.03	98.02	88.04	130	3.06	D		
2	87.06	230.08	212.07	202.08		7.10	N	1489.76	1471.77
3	70.07	327.17	309.15	299.14		1.16	P	1375.71	1357.71
4	101.07	455.19	437.18	427.19		2.22	Q	1278.69	1260.56
5	74.06	556.24	538.23	528.24		3.26	T	1150.64	1132.62
6	110.07	693.30	675.28	665.29		0.32	H	1049.60	1031.49
7	136.08	856.36	838.35	828.36		3.39	Y	912.52	894.51
8	136.08	1019.43	1001.43	991.43		6.45	Y	749.46	731.45
9	44.05	1090.47	1072.47	1062.46		7.49	A	586.44	568.29
10	72.08	1189.53	1171.51	1161.51		6.55	V	515.35	497.34
11	44.05	1260.56	1242.56	1232.57		7.59	A	416.29	398.28
12	72.08	1359.66	1341.62	1331.64		6.66	٧	345.24	327.17
13	72.08	1458.70	1440.20	1430.71		5.73	V	246.18	228.17
14	101.11						ĸ	147.11	129.10
			244 144 144 144 144 144 144 144 144 144	рекина рекин		у7 — Радни у	E b7-H20 b7 e01HYYAA/28 P01HYYAA/28		
		200 200 200 200 200 200 200 200 200 200	<sup>*,</sup> Билина 1900 500	600	007H 99 02H 99 04H 99 04H 99 700			02H+9V 02H+9V 90 10000	— РОТНУ Y AVAV — Б10-Н20 ь10
1:1 2	55-H20 100 200	┉╎╴╌┾╶┽╴╢╸╢┍╖	┍╌╌╍┥╙╻┛╦╝┍┑┨╍╴╌┑╟┙	ᠵᡪᠮᠯᡊᡃᡆᠲᡵᡫ᠊᠊᠋ᡎᡆᢈ	Ĩݭ╕ᢆ┉ᡰĹ᠊ᠯᢩᡰᡞᠬ	La Potter	5 b7.H20 b7 b7 c P QTH Y AV-28 c P QTH Y V AV-28 c P QTH Y V AV-28		— РОТНУ Y АИАИ — 510-Н20
: unue	55-H20 100 200	┉╎╴╌┾╶┽╴╢╸╢┍╖	┍╌╌┅न╢┼ <mark>╟╌╬</mark> ┧┍ᡪ┠╍╌╌┑╟┙	ᠵᡪᠮᠯᡊᡃᡆᠲᡵᡫ᠊᠊᠋ᡎᡆᢈ	Ĩݭ╕ᢆ┉ᡰĹ᠊ᠯᢩᡰᡞᠬ	La Potter	5 b7.H20 b7 b7 c P QTH Y AV-28 c P QTH Y V AV-28 c P QTH Y V AV-28		— РОТНУ Y АИАИ — Б10-Н20
Info Survey	100 200 X 2Y	┉╎╴╌┾╶┽╴╢╸╢┍╖	┍╌╌┅न╢┼ <mark>╟╌╬</mark> ┧┍ᡪ┠╍╌╌┑╟┙	ᠵᡪᠮᠯᡊᡃᡆᠲᡵᡫ᠊᠊᠋ᡎᡆᢈ	Ĩݭ╕ᢆ┉ᡰĹ᠊ᠯᢩᡰᡞᠬ	La Potter	5 b7.H20 b7 b7 c P QTH Y AV-28 c P QTH Y V AV-28 c P QTH Y V AV-28		— РОТНУ Y АИАИ — 510-Н20
: unue	100 200 X 2Y	┉╎╴╌┾╶┽╴╢╸╢┍╖	┍╌╌┅न╢┼ <mark>╟╌╬</mark> ┧┍ᡪ┠╍╌╌┑╟┙	ᠵᡪᠮᠯᡊᡃᡆᠲᡵᡫ᠊᠊᠋ᡎᡆᢈ	Ĩݭ╕ᢆ┉ᡰĹ᠊ᠯᢩᡰᡞᠬ	La Potter	5 b7.H20 b7 b7 c P QTH Y AV-28 c P QTH Y V AV-28 c P QTH Y V AV-28		— РОТНУ Y АИАИ — 510-Н20

The "Peptide ID List" shows each spectrum for which PEAKS found a matching peptide. Since there may be more than one spectrum that matches a peptide, these spectra would be listed together under a Hit node. Use the '+' and '-' boxes to expand and collapse the node to see the spectra that are listed together. With this dataset, spectra 4 and 5 can both be found under one hit. The "Peptide Alignment Panel" contains an "Ion Table", "Spectrum View Pane" and "Error Map" as was displayed in the "*De novo* View" seen above. For more information about these sections please refer to page 55.

Click on the "Protein View" tab on the upper left hand side. The following window will appear:

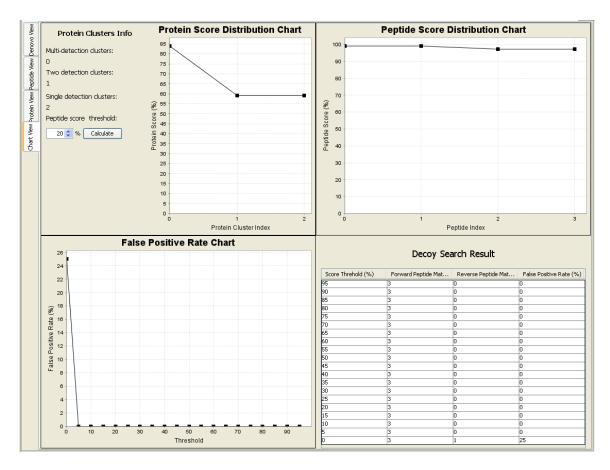
DB Search		ID	Mass	Displa	iy .	PEAKS(Score %)	Coverage(%)		Query matched	Marked		Description
										_		
	3 TRFE_BOVIN		2	36691.957			83.83 59.37	3.69 2.31		3		Serotransferrin - B Alcohol dehydroger
010000	opabrin_reabri		2	30091.937			33.37	2.01				Aconoracityaroge
equence B	rowser Sequen	ce Comparison										
	-	443 TRFE_BOVIN aining this sequence	from NCBI Entrez:									
Accession						Descript	ion					
Q29443 TF	RFE_BOVIN					Serotran	sferrin - Bos taurus (Bo	vine).				
Peptides	List:											
ID		Sequence	PEAKS(Score %)	RSD M/Z	Z	Mr(Calc)	Delta(Mass)	Error(ppm	) File	RT	Scan	Quality
Peptid	es ectrum 1	DNPQTHYYAVAVVK	99.0	0.0	802.91	2 16	03.7993 -0	.0061	3.8056 OrbiSample_new.		0.072	c
😑 🚺 не	: 4	HSTVFDNLPNPEDR	99.0			1	639.759					
	Spectrum 4 Spectrum 5		96.7 99.0	0.57	547.59 820.89	3 2		.0107	6.5511 OrbiSample_new. 3.9455 OrbiSample_new.		1.347 1.428	0
				EANKCAS FRENVLRI LVYEAGL KPNNLKPV								
51	SGPFVSCVKK	TSHMDCIKAI S	SNNEADAVTL DGG.	LVYEAGL KPNNLKPV	TV A							
51	SGPFVSCVKK	TSHMDCIKAI S	SNNEADAVTL DGG.		TV A							
51 101	SGPFVSCVKK EFHGTKDNPQ	TSHMDCIKAI S THYYAVAVVK F	NNEADAVTL DGG	LVYEAGL KPNNLKPV	TV <b>A</b>							
51 101 151	SGPFVSCVKK EFHGTKDNPQ AKLYKELPDP	TSHMDCIKAI S THYYAVAVVK F QESIQRAAAN F	SNNEADAVTL DGG (DTDFKLNEL RGK ?FSASCVPCA DQS	LVYEAGL KPNNLKPV KSCHTGL GRSAGWNI	TVA IPM DKC							
51 101 151 201	SGPFVSCVKK EFHGTKDNPQ AKLYKELPDP ACSNHEPYFG	TSHMDCIKAI S THYYAVAVVK F QESIQRAAAN F YSGAFKCLME G	SNNEADAVTL DGG CDTDFKLNEL RGK FFSASCVPCA DQS SAGDVAFVKH STV	LVYEAGL KPNNLKPV KSCHTGL GRSAGWNI SFPKLCQ LCAGKGTI	TVA IPM DKC LC							
51 101 151 201 251	SGPFVSCVKK EFHGTKDNPQ AKLYKELPDP ACSNHEPYFG GDNTRKSVDD	TSHMDCIKAI S THYYAVAVVK P QESIQRAAAN P YSGAPKCIME G YQECYLAMVP S	SNNEADAVTL DGG CDTDFKLNEL RGK ?FSASCVPCA DQS SAGDVAFVKH STV SHAVVARTVG GKE	LVYEAGL KPNNLKPV KSCHTGL GRSAGWNI SFPKLCQ LCAGKGTI FDKLPNP EDRKNYEI	TVA IPM JLC JLC							
51 101 151 201 251 301	SGPFVSCVKK EFHGTKDNPQ AKLYKELPDP ACSNHEPYPG GDNTRKSVDD KPDNFQLFQS	TSHMDCIKAI S THYYAVAVVK P QESIQRAAAN P YSGAFKCLME G YQECYLAMVP S PHGKDLLFKD S	SNNEADAVTI DGG (DTDFKLNEL RGK PFSASCVPCA DQS SAGDVAFVKH STV SHAVVARTVG GKE SAAGGELKIPS KMD	LVYEAGL KPNNLKPV KSCHTGL GRSAGWNI SPPKLCQ LCAGKGTI PDNLPNP EDRKNYEI DVIWELL NHAQEHFG	TVA IPM LC ILC ILR							

PEAKS 5 presents a list of proteins, ranked in descending order from highest score on downward. Clicking on any protein will display the peptides matched to that protein in the bottom pane.

In this case is Serotransferrin precursor from bovine. This protein has two matching peptides, which you can see in the "Peptide List". The entire sequence of the protein is shown and the matched parts are highlighted in blue. In this case the total matched part accounts for 3.69% of the protein.

Note that PEAKS 5 groups together homologous proteins which have the same peptide hits.

Click on the "Chart view" tab to see charts of the protein/peptide score distribution, the false positive rate and the decoy database search results. The following window will appear:



The graphs display protein and peptide scores as well as information on the false positive rate which is generated from the decoy database search. Please see page 61 for more information about the chart view.

#### 3.6 Run PTM Finder

Using the PTM finder, you can identify any additional PTMs and increase the coverage of the proteins that we have found. It is important to note that the PTM Finder can only be applied to a Protein ID results file. As it is very time consuming to run Protein ID with many PTMs, this allows searching for more PTMs in less time. Make sure that you click on a Protein ID result before performing a PTM Finder search.

1) Click the PTM Finder toolbar icon solution

Select "PTM Finder" from the "Tools" menu.

2) Enter the settings as shown:

PTM Finder					
ols	PTM Finder		Save Parameter	orbisample	~
	Mass Options			General Options	
ata Refine	Parent Mass Error Tolerance	0.1 Da 💙 Preci	ursor Mass Search Type:	Preprocess	this data 'on the fly'
e novo	Fragment Mass Error Toleran	ice: 0.8 Da 💙 💿 M	1onoisotopic 🔘 Average	Max Missed Cle	avages: 1 🗢
EAKS Search	Enzyme Options				
	Enzyme:	Trypsin		~	New Enzyme
PIDER Search	Digest Rule:	RK	{P}		Delete Enzyme
TM Finder		the above rule at both ends			Advance
uantification	PTM Options				
				Fixed Modification	
	Name Mono ma	la l	Add Fixed =>	Fixed Modification Indoacetic acid de	rivative
	4-hydroxynon 156.115	[CHK]	<= Remove	Togoarear aria de	in advo
	Homoserine -29.9928	[M]@C			
	Homoserine lac48.0034	[M]@C			
	Hydroxylation 15.9949	[PKDNRY]			
	ICPL-heavy 111.0416	[K], [X]@N			
	ICPL-light 105.0215	[K], [X]@N			
	Iodoacetic acid 58.0055	[C]			
	Lipoyl 188.033	[K]			
	Methyl ester 14.0156	[DE], [X]@C			
	Methylation 14.0156	[CKRHDENQ],			
	018 label 2.0042	[STY], [X]@C	Add Variable =>	Variable Modification	
	Propionamide 71.0371	[C]		Deamidation	
	Trimethylation 42.047	[CKRHDENQ],	<= Remove	• Oxidation M	
	Myristoylation 210.1984	[KC], [G]@N			
	N-acyl diglyceri 788.7258	[C]			
	N-isopropylcar 99.0684	[⊂]			
	N-Succinimidyl 127.0633	[K], [X]@N			
	Oxidation M 15.9949	[M]			
	Oxidation HW 15.9949	[HW]			
	Palmitoylation 238.2297	[CSTK]			
	Show unimod	New PTM		Max variable PTM per pe	ptide: 3 ᅌ
	Filter Options Filter the spectra which sa De novo amino acid score o Protein ID peptide score le		or use in the PTM search: mended 0.5 mended 0.65		
	<				

Saving the parameters for future reference is achieved by clicking on the "Save Parameter" button. For more information on setting up PTM Finder parameters see page 63. Click "OK" to commence analysis.

Peptides         99.0         1639,759           P          Htt 22         Htt 22         Htt 22         99.0         1639,759           O Spectrum 1         DepChinample.m         1.34/7         0.77           O Spectrum 3         AMELLINVK         99.0         0.14/8         20.08         2         0.00         0.0065         3.94550         Histore million         0.77         0.77         2         100.790         0.0061         3.94550         Histore million         0.77         0.77         2         100.790         0.0061         3.9450         Histore million         0.77         0.77         2         100.790         0.0062         6.0879         Orbidsample.m         0.365         0.77           Spectrum 3         AMELLINWK         97.29         0.0         057.3         2         1012.5916         0.0062         6.0879         Orbidsample.m         0.365         0.77           Spectrum 4         D         D-H         21.85         0.0         0.57.3         2         1012.5916         0.0062         6.0879         Orbidsample.m         0.314         0.77           Spectrum 3         AMELLINWK         97.29         0.0         155.09         H         1185.66         199.66 <th></th> <th></th> <th>Sequence</th> <th>PEAKS(S</th> <th>Score R</th> <th>SD</th> <th>M/Z</th> <th>Z</th> <th>Mr(Calc)</th> <th>Delta(Ma</th> <th>Error(ppm)</th> <th>File</th> <th>RT</th> <th>Scan</th> <th>Q.,</th>			Sequence	PEAKS(S	Score R	SD	M/Z	Z	Mr(Calc)	Delta(Ma	Error(ppm)	File	RT	Scan	Q.,
Spectrum 4         96.68         0.57         547.59         3         0.0         0.0107         6.5511         Orbisample.m         1.347         0.7           Spectrum 1         DNPQTHYYAVA         99.0         0.4         820.89         2         0.0         0.0065         3.9455         Orbisample.m         1.428         0.7           Spectrum 3         ANELLINWK         97.29         0.0         507.3         2         1012.5916         0.0062         6.0879         Orbisample.m         0.0365         0.77           Spectrum 1         TSDANINS]W         21.65         0.0         507.3         2         1012.5916         0.0062         6.0879         Orbisample.m         0.365         0.77           Spectrum 1         TSDANINS]W         21.65         0.0         507.3         2         1012.5916         0.0062         6.0879         Orbisample.m         0.365         0.77           Spectrum 2         TSDANINS]W         21.65         0.0         507.3         2         1012.5916         0.0062         6.0879         Orbisample.m         0.365         0.77           Petide Align         T         1380.67         1380.71         1480.66         1480.66         772.35	🔣 Peptid	les													
Spectrum 5 Spectrum 1         DNPQTHYVAV         99.0         0.14         820.89         2         0.0         -0.0065         3.9455Orbbiample.m         1.428         0.77           Spectrum 3         AMELINVK         97.29         0.0         5002.91         2         1603.7993         -0.0061         3.8056Orbbiample.m         0.072         0.77           Spectrum 3         AMELINVK         97.29         0.0         507.3         2         1012.5916         0.0062         6.0879Orbbiample.m         0.365         0.77           Spectrum 2         TSDAMIN[5]W         21.85         0.0         695.84         2         1389.6523         -0.0132         9.4669OrbiSample.m         0.365         0.77           Peptide Align          1         10.07         138.07         120.08         10.07         155.09         H          1         1485.70         1486.68         1487.68         752.35         13           3         74.06         326.15         308.14         298.15         343.17         T         1485.70         1486.68         1487.68         752.35         13           3         74.06         326.15         308.14         298.93.1         F         1216.56	🚊 🔘 Hi	t2	HSTVFDNLPNP.		99.0				1639.759						
Spectrum 1         DNPQTHYYAWA         99.0         0.0         802.91         2         1603.7993         -0.0061         3.8056         OrbiSample.m         0.07/2         0.77           Spectrum 3         ANELLINWK         97.29         0.0         507.3         2         1012.5916         0.0062         6.0879/OrbiSample.m         0.365         0.77           Spectrum 3         ANELINWK         97.29         0.0         697.32         2         1012.5916         0.0062         6.0879/OrbiSample.m         0.365         0.77           Spectrum 3         TSDANIN(5)W         21.85         0.0         695.84         2         1389.6523         -0.0132         9.4869/OrbiSample.m         0.314         0.77           Peptide Align         Mmonium         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y (2+)         #           1         110.07         138.07         120.08         110.07         155.09         H         1         1         1465.70         1486.68         1487.68         752.35         13           3         74.06         326.15         303.17         T         14165.70         1486.68         1499.66	(	) Spectrum 4			96.68	0.57	547.59	3	0.0	0.0107	6.5511	OrbiSample.m	1.34	7	0.7
Spectrum 3 Spectrum 3 ANELLINVK         APPL 29 97.29         0.0         507.3         2         1012.5916         0.0062         6.0879 6.0879         OrbiSample.m         0.365         0.77           Spectrum 2         TSDANIN(5)W         21.85         0.0         695.84         2         1389.6523         -0.0132         9.4669         OrbiSample.m         0.3614         0.77           Peptide Align         Immonium         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y(2+)         #           1         110.07         138.07         120.08         110.07         155.09         H         -         14         -         -         14           2         60.04         225.10         207.09         197.10         242.13         5         1503.71         1445.68         1497.66         752.35         13           3         74.06         326.15         308.14         298.15         343.17         T         1416.68         1399.65         1400.65         709.32         12           4         72.08         425.22         407.21         397.22         442.24         V         1319.55         1199.55         1199.53 </td <td></td> <td>) Spectrum 5</td> <td></td> <td></td> <td>99.0</td> <td>0.14</td> <td>820.89</td> <td>2</td> <td>0.0</td> <td>-0.0065</td> <td>3.9455</td> <td>OrbiSample.m</td> <td>1.42</td> <td>3</td> <td>0.7</td>		) Spectrum 5			99.0	0.14	820.89	2	0.0	-0.0065	3.9455	OrbiSample.m	1.42	3	0.7
Spectrum 3 Spectrum 2         ANEILINVK TSDANIN(5)W         97.29 21.85         0.0         507.3 2         2         1012.5916 1389.6523         0.0062 -0.0132         6.0879 9.4869/OrbiSample.m         0.38/5         0.7           Peptide Align         Immonium         b         b-H20         a         c         Seq         y         y-H20         z         z'         y(2+)         #           1         110.07         138.07         120.08         110.07         155.09         H         -         -         14         -         -         14           2         60.04         225.10         207.09         197.10         242.13         5         1503.71         1485.68         1487.68         782.35         13           3         74.06         326.15         308.14         298.15         343.17         T         1416.68         1399.65         1400.65         709.32         12         4         72.08         425.22         407.21         397.22         442.24         V         1315.63         1299.60         658.31         11           5         120.08         572.28         554.27         544.29         589.31         F         1216.56         1198.55         1299.60         6	O Sp	ectrum 1	DNPQTHYYAVA		99.0	0.0	802.91	2	1603.7993	-0.0061	3.8056	OrbiSample.m	0.07	2	0.7
Spectrum 2         TSDANIN[5]W         21.85         0.0         695.84         2         1389.6523         -0.0132         9.4869[orbliSample.m         0.31         4         0.77           Peptide Align         Immonium         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y (2+)         #           1         110.07         138.07         120.08         110.07         155.09         H         -	🛛 🖳 🔿 SF	ectrum 3	ANELLINVK		97.29	0.0	507.3	2	1012.5916	0.0062	6.0879	OrbiSample.m	0.36	5	0.7
Peptide Align         Immonium         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y (2+)         #           1         110.07         138.07         120.08         110.07         155.09         H           14         14           2         60.04         225.10         207.09         197.10         242.13         5         1503.71         1485.70         1486.68         1487.68         752.35         13           3         74.06         326.15         308.14         298.15         343.17         T         1416.68         1398.66         1399.65         1400.65         709.32         12           4         72.08         425.22         407.21         397.22         442.24         V         1315.63         1297.62         1298.60         1299.60         658.31         11           5         120.08         572.28         599.31         F         1216.56         1198.51         199.53         120.05         593.24         704.34         D         1069.49         1051.48         1052.46         1053.46         535.25         9           7         87.06         801.37         783.34	🛛 🔾 Sp	ectrum 3	ANEILINVK		97.29	0.0	507.3	2	1012.5916	0.0062	6.0879	OrbiSample.m	0.36	5	0.7
#         Immonium         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y(2+)         #           1         110.07         138.07         120.08         110.07         155.09         H           1           2         60.04         225.10         207.09         197.10         242.13         5         1503.71         1485.70         1486.68         1487.68         752.35         13           3         74.06         326.15         308.14         298.15         343.17         T         1416.68         1399.65         1490.65         709.32         12           4         72.08         425.22         407.21         397.22         442.24         V         1315.63         1297.62         1298.60         1299.60         658.31         11           5         120.08         572.25         554.27         544.29         589.31         F         1216.56         1199.55         1199.53         1200.53         608.78         10           6         88.04         681.37         783.34         773.36         818.38         N         954.46         936.45         937.44         938.44	<u> </u>	pectrum 2	TSDANIN[5]W.		21.85	0.0	695.84	2	1389.6523	-0.0132	9.4869	OrbiSample.m	0.31	1	0.7
1       110.07       138.07       120.08       110.07       155.09       H       Image: constraint of the state of the	Peptide	Align													
1       110.07       138.07       120.08       110.07       155.09       H       Image: constraint of the state of the	#	Immonium	Ь	Ь-Н2О	а	с	-	Seq	y	y-H2O	z	z'	y (2+)	#	÷
2       60.04       225.10       207.09       197.10       242.13       5       1503.71       1485.70       1486.68       1487.68       752.35       13         3       74.06       326.15       308.14       298.15       343.17       T       1416.68       1399.65       1400.65       709.32       12         4       72.08       425.22       407.21       397.22       442.24       V       1315.63       1297.62       1298.60       1299.60       658.31       11         5       120.08       554.27       544.29       599.31       F       1216.56       1198.55       1300.54       608.78       100         6       88.04       687.32       669.30       659.32       704.34       D       1069.49       1051.48       1052.46       1053.46       535.25       9         7       87.06       801.37       783.34       773.36       818.38       N       954.46       936.45       937.44       938.44       477.73       8         8       86.10       914.44       896.41       931.46       L       840.42       822.41       823.39       824.39       420.71       7         9       70.07       1011.49	1														
3         74.06         326.15         308.14         298.15         343.17         T         1416.68         1398.66         1399.65         1400.65         709.32         12           4         72.08         425.22         407.21         397.22         442.24         V         1315.63         1297.62         1298.60         1299.60         658.31         11           5         120.08         572.28         554.27         544.29         589.31         F         1216.56         1198.55         1199.53         1200.53         608.78         10           6         88.04         687.32         669.30         659.32         704.34         D         1069.49         1051.48         1052.46         1053.46         535.25         9           7         9         70.07         1011.49         933.44         477.73         8         8         86.10         914.44         896.41         886.44         931.46         L         840.42         822.41         823.39         824.39         420.71         7           9         70.07         1011.49         993.48         983.49         1028.52         P         727.33         709.33         710.31         711.31         364.17									1503.7	1 1485.7	0 1486.68	1487.68	752.3		
4       72.08       425.22       407.21       397.22       442.24       V       1315.63       1297.62       1298.60       1299.60       658.31       11         5       120.08       572.28       554.27       544.29       589.31       F       1216.56       1198.55       1199.53       1200.53       608.78       10         6       88.04       687.32       669.30       659.32       704.34       D       1069.49       1051.48       1052.46       1053.46       535.25       9         7       87.06       801.37       783.34       773.36       818.38       N       954.46       936.45       937.44       938.44       477.73       8         86.10       914.44       896.41       886.44       931.46       L       840.42       822.41       823.93       824.39       420.71       7         9       70.07       1011.49       993.48       983.49       1028.52       P       727.33       709.33       710.31       711.31       364.17       6         10       87.06       1125.53       1107.52       1097.54       1142.56       N       630.28       612.27       613.26       614.26       315.64       5 <t< td=""><td></td><td></td><td></td><td></td><td></td><td>_</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>						_									
5         120.08         572.28         554.27         544.29         589.31         F         1216.56         1198.55         1199.53         1200.53         608.78         10           6         88.04         667.32         669.30         659.32         704.34         D         1069.49         1051.48         1052.46         1053.46         535.25         9           7         87.06         801.37         783.34         773.36         818.38         N         954.46         936.45         937.44         938.44         477.73         8           8         86.10         914.44         896.41         886.44         931.46         1         840.42         822.41         823.39         824.39         420.71         7           9         70.07         1011.49         993.48         983.49         1028.52         P         727.33         709.33         710.31         711.31         364.17         6           10         87.06         1125.53         1107.52         1097.54         1142.56         N         630.28         612.27         613.26         614.26         315.64         5           11         70.07         1222.59         1204.58         1194.59 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>															
6       88.04       687.32       669.30       659.32       704.34       D       1069.49       1051.48       1052.46       1053.46       535.25       9         7       87.06       801.37       783.34       773.36       818.38       N       954.46       936.44       938.44       477.73       8         8       86.10       914.44       896.41       886.44       931.46       L       840.42       822.41       823.39       824.39       420.71       7         9       70.07       1011.49       993.48       983.49       902.852       P       727.33       709.33       710.31       711.31       364.17       6         10       87.06       1125.53       1107.52       1097.54       1142.56       N       630.28       612.27       613.26       614.26       315.64       5         11       70.07       1222.59       1204.58       1194.59       1239.61       P       516.25       499.20       500.21       258.62       4         12       102.06       1351.63       1333.62       1323.63       1368.66       E       419.19       401.18       402.16       403.16       210.09       3       3         <															
7       87.06       801.37       783.34       773.36       818.38       N       954.46       936.45       937.44       938.44       477.73       8         8       86.10       914.44       896.41       886.44       931.46       L       840.42       822.41       823.39       824.39       420.71       7         9       70.07       1011.49       993.48       983.49       1028.52       P       727.33       709.33       710.31       711.31       364.17       6         10       87.06       1125.53       1107.52       1097.54       1142.56       N       630.28       612.27       613.26       614.64       315.64       5         11       70.07       1225.59       1204.58       1194.59       1239.61       P       516.25       499.20       500.21       258.62       4         12       102.06       1351.63       1333.62       1323.63       1368.66       E       419.19       401.18       402.16       403.16       210.09       3         13       98.04       1466.66       1448.64       1438.68       D       290.14       272.14       273.12       274.12       145.57       2         14															
8         86.10         914.44         896.41         886.44         931.46         L         840.42         822.41         823.39         824.39         420.71         7           9         70.07         1011.49         993.48         983.49         1028.52         P         727.33         709.33         710.31         711.31         364.17         6           10         87.06         1125.53         1107.52         1097.54         1142.56         N         630.28         612.27         613.26         614.26         315.64         5           11         70.07         1222.59         1204.58         1194.59         1239.61         P         516.25         498.23         499.20         500.21         258.62         4           12         102.06         1351.63         133.62         132.63         1368.66         E         419.19         401.18         402.16         403.16         210.09         3           13         88.04         1466.66         1448.64         1438.68         D         290.14         272.14         273.12         274.12         145.57         2	7					_									
9         70.07         1011.49         993.48         983.49         1028.52         P         727.33         709.33         710.31         711.31         364.17         6           10         87.06         1125.53         1107.52         1097.54         1142.56         N         630.28         612.27         613.26         614.26         315.64         5           11         70.07         1222.59         1204.58         1194.59         1239.61         P         516.25         498.23         499.20         500.21         258.62         4           12         102.06         1351.63         1333.62         1323.63         1368.66         E         419.19         401.18         402.16         403.16         210.09         3           13         88.04         1466.66         1448.64         1438.68         D         290.14         272.14         273.12         274.12         145.57         2	8	86.10	914.44	896.41		93	31.46	L	840.42	822.41	823.39	824.39	420.7	1	7
11       70.07       1222.59       1204.58       1194.59       1239.61       P       516.25       498.23       499.20       500.21       258.62       4         12       102.06       1351.63       1333.62       1323.63       1368.66       E       419.19       401.18       402.16       403.16       210.09       3         13       88.04       1466.66       1448.64       1438.66       1483.68       D       290.14       272.14       273.12       274.12       145.57       2         14       100.04       1466.66       1448.64       1438.66       1483.68       D       290.14       272.14       273.12       274.12       145.57       2         14       100.04       1486.66       1448.64       1438.66       160.02       15	9	70.07		993.48	983.49			Р	727.33				364.1	7	6
12       102.06       1351.63       1333.62       1323.63       1368.66       E       419.19       401.18       402.16       403.16       210.09       3         13       88.04       1466.66       1448.64       1438.66       1483.68       D       290.14       272.14       273.12       274.12       145.57       2         400.16       1466.66       1448.64       1438.66       1483.68       D       290.14       272.14       273.12       274.12       145.57       2         100.00       100	10	87.06	1125.53	1107.52	1097.54	11	42.56	N	630.28	612.27	613.26	614.26	315.6	4	5
13 88.04 1466.66 1448.64 1438.66 1483.68 D 290.14 272.14 273.12 274.12 145.57 2	11	70.07	1222.59	1204.58	1194.59	12	39.61	Р	516.25	498.23	499.20	500.21	258.6	2	4
	12	102.06	1351.63	1333.62	1323.63	13	68.66	Е	419.19	401.18	402.16	403.16	210.0	9	3
P2			1466.66	1448.64	1438.66	14	83.68	D							2
							y6								
		· · · ·	200	₹20× 400	- 44 NH3 5 b10- NH3[2+] 00-	بآبي	*****	24 	بي بدي 10	. <b></b>	1200	1400		1600	
0 0	jun alla		200	<del>╷╄╍<sub>┫</sub>╝┑</del>	<del>∙╅╄╺┉╇┖╍</del> ╈	بآبي	*****		44 <u>4</u>	<mark>, , , , , , , , , , , , , , , , , , , </mark>	1200	1400 0		1600 0	
Info     Survey     Alignment     Error Map	:	1:1   2X   2Y	200 ,	<del>╷╄╍<sub>┫</sub>╝┑</del>	<del>∙╅╄╺┉╇┖╍</del> ╈	بآبي	*****		44 <u>4</u>	- <b>.</b>	1200				
	Info S	1:1 2X 2Y 5urvey Alignm	200 ,	<del>╷╄╍<sub>┫</sub>╝┑</del>	<del>∙╅╄╺┉╇┖╍</del> ╈	بآبي	*****		44 <u>4</u>	- <del>, , , , , , , , , , , , , , , , , , ,</del>	1200				

After the PTM Finder search is com	plete, the "Peptide	View" window will appear:
------------------------------------	---------------------	---------------------------

The results will be displayed in the same format as was seen for Protein ID. Recall that the Protein ID search identified spectra 1, 3, 4 and 5. The PTM finder search also displayed spectra 2 with the addition of deamidation on N.

#### 3.7 Run an inChorus Search

Performing the search with the same data by different search engines is useful both for finding new proteins and confirming others. You can perform and inChorus search using PEAKS Protein ID, X!Tandem, OMSSA, Mascot and Sequest. For this example we will be performing a local search using the X!Tandem and OMSSA search engines. If you have not already set up your search engine preferences, see page 93 for more instructions.

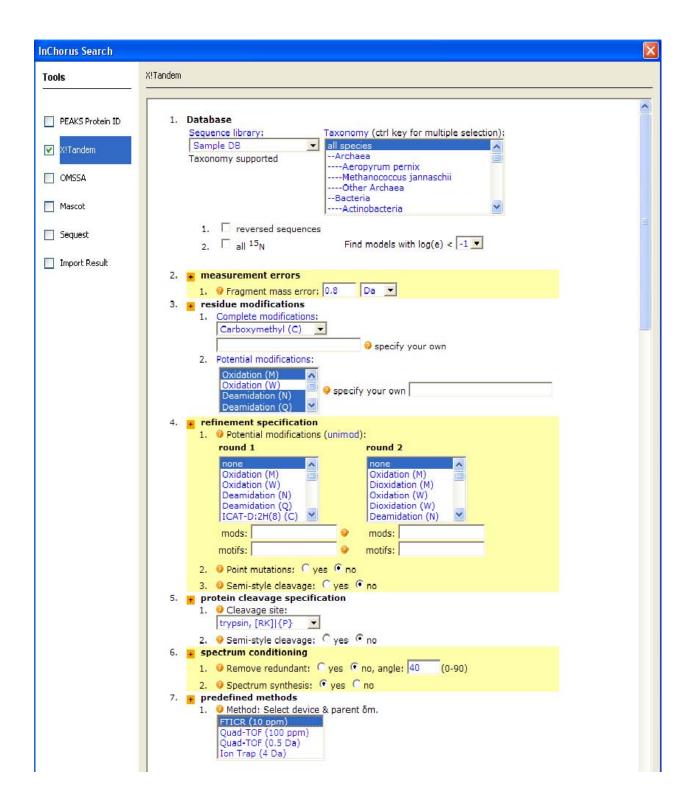
- 1) Click on the orbisample.mzxml file
- 2) Then click the inChorus Search toolbar icon Or

Select "inChorus Search" from the "Tools" menu.

The inChorus search window will open. Check the "PEAKS Protein ID" box and select the name. Enter the following settings:

InChorus Search	
Tools	PEAKS Protein ID
PEAKS Protein ID	Database Search Save Parameter orbisample
<ul> <li>X!Tandem</li> <li>OMSSA</li> </ul>	Mass Options       General Options         Parent Mass Error Tolerance:       0.1       Da           Parent Mass Search Type:       Preprocess this data 'on the fly'
Mascot	Fragment Mass Error Tolerance:       0.8       Da       Monoisotopic       Average       Max Missed Cleavages:       1         Enzyme Options       Image: Cleavage Options       Image: Cleavage Options       Image: Cleavage Options       Image: Cleavage Options
Sequest	Enzyme: Trypsin New Enzyme
Import Result	Digest Rule:     RK     {P}     Delete Enzyme       Image: Prind peptides that satisfythe above rule at both ends     Advance
	PTM Options
	Name         Mono mass         Residue site         Add Fixed =>         Fixed Modification           Citrullination         0.984         [R]         Image: Citrullination         Image: Citrul C
	C.truilination 0.984 [R] C-Mannosylation 162.0528 [W] Deamidation 0.984 [NQ]
	Dehydration -18.0106 [YTS], [NQ]@C
	Dioxidation 31.9898 [YMRKPCF]
	Flavin adenine 783.1415 [CHY]
	Farnesylation       204.1878       [C]         Show unimod       New PTM       Max variable PTM per peptide:       3 🗘
	Database Options
	Select database     Select Database: Sample D8     New Database     Set Taxa
	Advanced Options PEAKS uses a hybrid search technique that requires some sequence tags to help in the search
	<ul> <li>Run de novo using different parameters than the above</li> <li>Run de novo using the same parameters as above (default)</li> <li>Validation - decoy search</li> </ul>

Next, check the "X!Tandem" box and select the name. Note that you will need to use the "Ctrl" button to select multiple search engines. Enter the following settings:



InChorus Search Tools OMSSA ~ PEAKS Protein ID NCBI X!Tandem Search Status Download MSSA Search FAO Browse Mascot Enzyme: Trypsin • Maximum missed cleavages: 3 💌 Sequest Species to search (ctrl key for multiple selection): all species Import Result Sequence library -Aeropyrum pernix Sample DB --Methanococcus jannaschii Taxonomy supported --- Other Archaea -Bacteria --Actinobacteria Hitlist max length: 10 💌 E-value cutoff: 1 -Fixed mods (ctrl key for multiple selection): Variable mods (ctrl key for multiple selection): carboxyamidomethylation of K citrullination of R carboxykynurenin of W deamidation of N and Q carboxymethyl C citrullination of R dehydro of S and T di-018 on peptide n-term deamidation of N and Q di-iodination of Y di-methylation of K dehydro of S and T di-O18 on peptide n-term Maximum variable mod combinations searched per peptide: 64 🗾 Precursor mass tolerance (Da): 0.1 Product mass tolerance (Da): 0.8 Precursor mass search type: monoisotopic Product mass search type: monoisotopic -Lower bound of precursor charge: 1 Upper bound of precursor charge: 3 -Minimum charge to start using multiply charged products: Fraction of product peaks below precursor to determine +1 precursor: 0.95 Peak intensity cutoff: Number of top intensity peaks in first pass: 0 (fraction of most intense) 6 🔻 Ions to search 1: b -Ions to search 2: y 💌 < > OK Cancel Help

Finally, check the "OMSSA" box and select the name. Enter the following settings:

Click the "Ok button. When the inChorus search is complete you should see the following new additions in the "Project View" panel:

PEAKS 1 [21-Jan-09 11:38]
 XTANDEM 2 [21-Jan-09 11:39]
 OMSSA 3 [21-Jan-09 11:40]
 INCHORUS 4 [21-Jan-09 11:40]

Presented here are individual reports for PEAKS, X!Tandem and OMSSA as well as an inChorus report that compares the individual reports. To see each of these reports, click on the report that you would like to see in the "Project View" panel.

ID	Sequence	PEAKS(Score %)	RSD	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(p	File	RT	Scan	Qu
😺 Peptides												
	DNPQTHYYAVAVVK	99.0	0.0	802.91	2	1603.7993	-0.0061	3.8056	OrbiSample.mz	0.07	2	0.788
Spectrum 2	TSDANIN[3]WNNLK	99.0	0.0	695.84	2	1389.6523	-0.0132	9.4869	OrbiSample.mz	0.31	4	0.785
Spectrum 3	ANELLINVK	97.38	0.0	507.3	2	1012.5916	0.0062	6.0879	OrbiSample.mz	0.36	5	0.781
	ANEILINVK	97.38	0.0	507.3	2	1012.5916	0.0062	6.0879	OrbiSample.mz	0.36	5	0.781
Hit 4	HSTVFDNLPNPEDR	99.0				1639.759						
Spectrum 5		99.0	0.14	820.89	2	0.0	-0.0065	3.9455	OrbiSample.mz	1.42	8	0.787
Spectrum 4		96.64	0.57	547.59	3	0.0	0.0107	6.5511	OrbiSample.mz	1.34	7	0.777

The "Peptide View" results for the PEAKS Protein ID search can be seen below:

The "Peptide View" results for the X!Tandem search can be seen below:

lew	ID	Sequence	XTANDEM(E-value)	RSD	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(ppm)	File	RT	Scan	Q
h ا	😡 Peptides												
Ĕ	Spectrum 1	DNPQTHYYAVAVVK	1.9E-5	-	802.91	2	1603.7993	-0.0061	3.8056	OrbiSample.mz	0.07	2	0.788
å)	Spectrum 5	HSTVFDNLPNPEDR	1.2E-4	-	820.89	2	1639.759	-0.0065	3.9455	OrbiSample.mz	1.42	8	0.787

"Peptide View" results for the OMSSA search can be seen below:

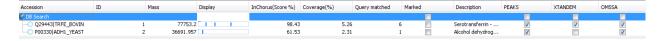
lev	ID	Sequence	OMSSA(E-value)	RSD	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(ppm)	File	RT	Scan	Quality
- A	🗟 Peptides												
eptic	Spectrum 1	DNPQTHYYAVAVVK	1.17E-10	-	802.91	2	1603.7993	-0.0061	3.8056	OrbiSample.mzX	0.07	2	0.788
a	Spectrum 2	TSDANIN[4]WN	0.0816	-	695.84	2	1389.6484	-0.0171	12.2978	OrbiSample.mzX	0.31	4	0.785
	Spectrum 5	HSTVFDNLPNPEDR	6.75E-6	-	820.89	2	1639.759	-0.0065	3.9455	OrbiSample.mzX	1.42	8	0.787

The inChorus report contains most of the information that is seen in a PEAKS Protein ID results file (page 55). Click on the "Peptide View" tab:

ID	Sequence	InChorus(	11	M/Z	Z	Mr(Calc)	Delta(	Error(	Ļ	RT	Scan	Qua	PEAKS(	XTANDEM(	OMSSA(E
😺 Peptides						- 1			Т						
	DNPQTHYYAVAVVK	99.98	- 8	302.91	2	1603.7993	-0.0061	3.8056.		0.07	2	0.788	99.0	1.9E-5	1.17E-10
Spectrum 3	ANELLINVK	97.38	-	507.3	2	1012.5916	0.0062	6.0879.	••	0.36	5	0.781	97.38	-	
	HSTVFDNLPNPEDR	96.64	- 5	547.59	3	1639.759	0.0107	6.5511.	-	1.34	7	0.777	96.64	-	
🚊 🛞 Hit 5	TSDANIN[3]WNNLK	99.97				1389.6523									
Spectrum 2		99.85	- 6	595.84	2	0.0	-0.0132	9.4869.		0.31	4	0.785	99.0	-	0.0816
Spectrum 5		99.97	- 8	320.89	2	0.0	-250.1132	152529.83.		1.42	8	0.787	99.0	1.2E-4	6.75E-6
Spectrum 3	ANEILINVK	97.38	-	507.3	2	1012.5916	0.0062	6.0879.		0.36	5	0.781	97.38	-	-

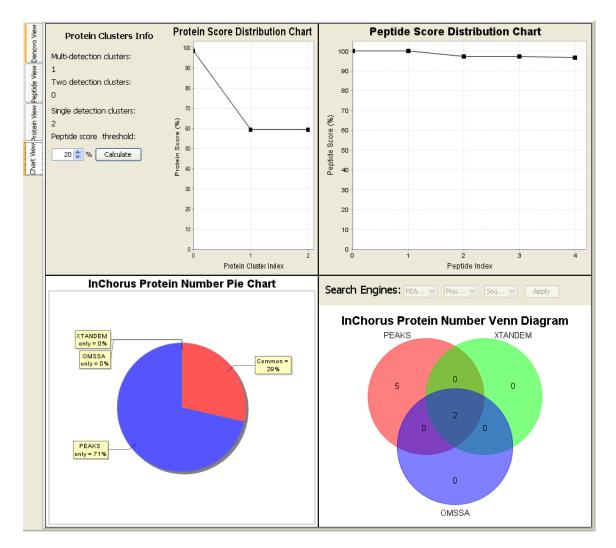
The "Peptide View" of an inChorus report contains the scores received by each search engine involved in the inChorus search. A "–" indicates that the search engine did not find that a protein sequence for that particular spectrum. Notice that while PEAKS Protein ID found spectra 1-5, X!Tandem found 1, 5 and OMSSA found 1, 2, 5.

Click on the "Protein View" tab:



The "Protein View" of an inChorus report displays the proteins that were found and indicates by checkmarks whether the different search engines found that protein or not. In this case, the X!Tandem and OMSSA searches did not generate any extra results that PEAKS did not find but helped to confirm that the first protein is good match.

Click on the "Chart View" tab:



The top two charts, the "Protein Score Distribution Chart" and the "Peptide Score Distribution Chart" are in the same format to those that are seen in the "Chart View" of a PEAKS Protein ID search (page 61). More information about the "inChorus Protein Number Pie Chart" and the "inChorus Protein Number Venn Diagram" can be found on page 72.

#### 3.8 Perform a SPIDER Search

In this example, spectrum 6 has not been identified with database searching tools. In order to gain more information from our data, we will run a SPIDER search next. For more information about the SPIDER search refer to page 63.

- 1) Click on the OrbiSample.mzxml file
- Next, click the SPIDER Search toolbar icon *Or* Select "SPIDER Search" from the "Tools" menu.
- 3) Enter the settings as shown:

SPIDER Search	
ools	SPIDER Search Save Parameter
	Query Options
Data Refine	Segment Match Non-gapped Homology Match
De novo	
	General Options
PEAKS Search	Mass Tolerance (Da): 0.1 Report Top: 1
SPIDER Search	Leucine equals Isoleucine 📝 Lysine equals Glutamine 📝
PTM Finder	PTM Options
D	Name Mono mass Residue site Add Fixed =>
uantification	Litruination U.984 [K] . Iodoacetic acid derivative
	C-Mannosylation 162.0528 [W]
	Deamidation 0.984 [NQ]
	Dehydration -18.0106 [YTS], [NQ]@C Add Variable => U Variable Modification
	Dimethylation 28.0313 [CKRHDENQ],
	Dioxidation 31.9898 [YMRKPCF] = Remove Deamidation
	Flavin adenine 783.1415 [CHY]
	Show unimod New PTM Max variable PTM per peptide: 4
	Database Options
	Select Database: SampleDB
	New Database Edit Database
	Set Taxa
	OK Cancel Help

Parameters can be saved for future reference by clicking on the "Save Parameter" button. For more information on setting up SPIDER Search parameters see page 68. Click "OK" to commence analysis.

After the HE\_NEW.HOME . . SPICIER 5 [28-Jan-09 14:50] . PEAKS 1 [28-3an-09 14:47] # PEAKS 13 [29-3an-09 15:14] # IND-OPUS 4 [28-3an 00 14:47) × 01 **SPIDER** search has 0.0 CVN(2)\_M2VPQ(2)N(2) completed, the "Peptide 1 View" 117,10 232,11 347,10 510,21 638,20 767,31 928,30 1291,4 1225,5 82.67 197.69 312.12 475.11 403.24 732.23 893.30 215. 200 483. 483. 481. 900. 911. 109.9 994.9 831.8 703.6 window will appear. The format is 11 identical to 258.14 222.48 173.11 123.58 88.06 what was seen 427.2 128.2 128.1 345.2 246.3 176.1 in the results of a Protein ID search: 100 11 21 21 Defa 1 Algerert. EnarM Saver 600

Note that all spectra can now be identified by the SPIDER search. Spectrum 6 is well identified.

Clicking on the "Peptide details" tab will display the protein with its matched peptides in red. SPIDER will also display a reconstructed sequence. See page 66 for more information.

~		ISDANLINWINNER		26.0	0.08	695.84	
View	Spectrum 3	ANELLLNVK		22.0	0.0	507.3	
	Spectrum 4	LSHEFALNGNPQNP		14.0	0.57	547.59	
Peptide		HSTVFDNLPNPEDR		32.0	0.14	820.89	
	🛄 💭 Spectrum 6	VDDYQEC[1]YLAMVPSHAVVA	AR .	23.0	0.5	798.37	
Protein View	Peptide Align Peptid Select peptides for disp	e Details		Q2	9443 TRFE	E_BOVIN	]
	Denovo W	(CarboxymethylC)W	<fwl></fwl>	YLAMVPSHA []	RLIR		

	Denovow (Carboxymethylc) w <fwl> YLANVPSHA[RL]R</fwl>	
	Recon AD (CarboxymethylC) W <re(carboxymethylc)>YLAMVP5HA[VVA]R</re(carboxymethylc)>	
	+	
	Matched peptides shown in blue, SPIDER matches shown in red	
1	MRPAVRALLA CAVLGLCLAD PERTVRWCTI STHEANKCAS FRENVLRILE	
51	SGPFVSCVKK TSHMDCIKAI SNNEADAVTL DGGLVYEAGL KPNNLKPVVA	
101	EFHGTKDNPQ THYYAVAVVK KDTDFKLNEL RGKKSCHTGL GRSAGWNIPM	
151	AKLYKELPDP QESIQPAAAN FFSASCVPCA DQSSFPKLCQ LCAGKGTDKC	
201	ACSNHEPYFG YSGAFKCLME GAGDVAFVKH STVFDNLPNP EDRKNYELLC	
251	GDNTRKSVDD YQECYLAMVP SHAVVARTVG GKEDVIWELL NHAQEHFGKD	

## Chapter

#### 4. Load data

#### 4.1 Data Format

Before loading data files into PEAKS, you must make sure that the data is in an accessible format. PEAKS handles data files in the following formats:

.PKL: The file format associated with MassLynx software.

.DTA: The file format associated with SEQUEST software

.MGF: The file format associated with Mascot software.

.ANZ - the zip compressed XML based file format associated with PEAKS 4.5

.XML format files using the mzXML schema

.XML format files using the mzData schema

.RAW files from Thermo Electron instruments

.WIFF files from ABI/Sciex QSTAR and QTRAP instruments

.RAW files from Waters QTOF instruments

.BAF, .YEP and folders of .FID files from Bruker instruments

.D files from Agilent QTOF instruments

.DAT files created by BSI's ABI converter software

PEAKS 5 project

#### 4.2 Data Conversion

It is best to import RAW data directly, so that PEAKS can access the complete, unprocessed experimental data including the MS survey scan and retention time information. This will ensure that the PEAKS analysis does not suffer from poor preprocessing.

In order to load RAW data from different vendors, PEAKS may require third-party software to be installed. Please consult the following instructions for third-party software requirements.

#### Thermo Data

RAW data from Thermo Electron mass spectrometers can be loaded, provided that the XCalibur software is installed on the same computer as PEAKS 5.

#### Agilent Data

PEAKS 5 can load native data from Agilent QTOF, provided that the MassHunter software is installed on the same computer.

#### Bruker Data

PEAKS 5 can load data from Bruker mass spectrometers provided that the CompassXport software is installed on the same computer. If loading .fid files, which are stored in a network of folders, select the top level folder to load them all at once.

#### Shimadzu Data

Shimadzu mass spectrometer data can be loaded, provided that the Shimadzu software is installed on the same computer as PEAKS 5.

#### Applied Biosystems Data

WIFF data from Applied Biosystems/Sciex QSTAR (or QTRAP) mass spectrometers can be loaded, provided that the Analyst QS (Analyst 1.4.1 for QTRAP) software and the MSX plug-in are installed on the same computer as PEAKS 5. The MSX tool is produced and sold by Infochromics Ltd., and is available (at cost) from Bioinformatics Solutions Inc. Please contact a BSI sales representative to obtain an evaluation or full license.

#### Varian Data

A conversion tool is embedded into Varian's data acquisition software which allows the conversion of Varian raw data into .pkl files which can be immediately read by PEAKS.

The .trans type data (raw) is converted in Varian programs by clicking "File", "Save As" and selecting the .pkl file format or by clicking "File", right clicking "Export" and selecting ".pkl". If you are viewing a chromatogram with the Varian software, all the spectra data in the viewed chromatogram is converted to the .pkl format. Likewise, if you are viewing a single spectrum and choose to convert the data, only the viewed spectra will be converted.

#### Waters/Micromass (MassLynx) Data

PEAKS 5 can import RAW data from Waters/MicroMass QTOF instruments using a utility called wolf.exe (originally created as part of the Sashimi Project) to access MassLynx libraries and convert the data. PEAKS provides a version of wolf.exe compatible with MassLynx 4.1. If you need a different version of wolf.exe, please visit:

www.bioinfor.com/products/peaks/support/watersmicromass.php

Additionally, you must make sure that the following MassLynx libraries are installed on the same computer as PEAKS and wolf.exe:

- DACServer.dll
- Genutil.dll
- MetaGD32.dll
- raw.dll
- securityAccess.dll
- securitySettings.dll
- securitySignature.dll

#### ABI 4700 or 4800 Data

BSI has created a converter to extract the data from an ABI-Oracle database. If you require this separate, free tool, contact your sales representative. Once installed, you can start up the ABI 4700 Data Extractor from the Start menu.

#### System Requirements

This extractor can be installed on the same machine as ABI 4700 Explorer and the Oracle database (we will call this machine the 4700 SERVER in the following instructions) or another machine that has direct network access (no firewall or proxy required) to the 4700 SERVER. Windows 2000 or Windows XP is recommended for use with this tool.

#### Configuration

Before using the ABI 4700 Data Extractor, it must be configured. To do so, choose "Settings" from the "File" menu. Configuration requires the following:

4700 SERVER Name or IP Address: input "localhost" if the Extractor is running on the 4700 SERVER (this is the default value), otherwise enter the IP address of the 4700 SERVER.

The socket used by the 4700 SERVER: this is the port that the Oracle database listens to (the default is 1521).

Username to access the Oracle database: most likely we do not need to change this (the default is "tsquared").

Password to access the Oracle database: mostly likely you do not need to change this one either.

#### Data extraction procedure

1. Load Spot Set List from the database: (Do it via menu File | Load Spot Set List). The extractor will export the peak list of a spot set into a PKL file.

2. Open a Spot Set: (menu File | Open Spot Set) Spot Set Chooser will help the user to choose a spot set. After selecting a spot set, click 'OK' to open it. The job run information of a spot set will be shown.

3. Select a job run: There is a button to select before each job run. Only the MS/MS job run can be selected for export, as the precursor information is needed. Select a job run and click 'Convert' to do the extraction.

4. Choose a filename to save: After clicking the 'Convert' button, the user needs to input a file name and the peak lists of the selected job run will be exported.

#### 4.3 Load a New File

After making sure that you have the appropriate third party software, use the instructions below to load the data files.

1) Select "New File" from the file menu or use the blue file menu icon **o**]. First select the mass spectrometry vendor from the drop down menu or keep the default "General" setting.

2) Select your instrument from the drop down menu. If you selected the "General" setting in the option above, the instrument names will also be general, however if you selected a particular vendor, the vendor specific instrument names will be displayed. If you do not see the instrument that you used, you click on the "Add Instrument" button to create a new instrument.

3) Finally, browse your computer to locate the file to be processed and click open. The file will now begin loading.

iteps	Open Files						
<ol> <li>Select Manufacturer</li> <li>Select Instrument</li> <li>Select File(s)</li> </ol>		New Project 1 Thermo Scientific LTQ FT Ultra Hyb			•	Add Instrument	
	Look in:	sampleDa	ta		•]	🤌 📁 💷 📰	
	Recent Items	BSA_dig_	<u>L30_06.raw</u>				
	Documents						
	Network						
		File name: Files of type:	.raw (Thermo Xcalibur	raw File)			Open ancel

#### 4.4 Create a New Project

2)

1) To create a new project, select "New Project" from the file menu or using the "New project" icon on the toolbar. The "Project Properties" window will open.

Create a name	📐 New Project		X
for your project and click	Steps	Project Properties	
browse to select the location of the data for that project. You can use the notes and	<ol> <li>Project Properties</li> <li></li> </ol>	Project Name: Project Location: Project Folder: Notes/Description: Example: Sample	Project 1 C:\Peaks Studio 5.0\.\derbyServer\serverDB C:\Peaks Studio 5.0\.\derbyServer\serverDB\Project 1 es from Orbitrap on August 10-12
description box, to remind yourself of information specific to the project.		Type and organization of Basic Project Several non-labelled	samples for comparison (each sample can be fractionated)
			< <back next="">&gt; Cancel Help</back>

"Sample 3) The Properties" window will open. Give your sample a You can name. now select file/s that pertains to this sample using the "Add a file for this sample". То remove files or clear the list, you can use the "Remove from list" and "Clear list" buttons respectively. You may also leave

Steps	Sample Propertie	25			
I. Project Properties 2. Sample Properties	Give this sample a n Select files	ame:	iample 1		
3	Name	Size		Date Modified	Туре
2	on\data\iain\26 on\data\iain\26			03/02/2006 10:05:00 AM 03/02/2006 13:18:00 PM	ra ra
	Add a file for Notes about this sa		Remov	ve file from list	Clear list
			Add another	sample Rem	ove current sample

notes about the sample for reference.

- 4) To add another sample or remove the current sample, use the "Add another sample" and "Remove current sample" buttons, respectively.
- 5) Select the "Next" button once all relevant files are added to each sample.
- 6) The "Instrument Details" window will open. Select the instrument that was used to generate the experimental data. From the drop-down menu, select "General" for the common instrument types (example: FT-TRAP) or specify the instrument vendor and chose the vendor specific instrument type (example: LCQ Ion Trap). Hold down the Ctrl key to select additional instruments. Notice that when you select the instrument type, the default

parameters will be displayed the right har pane. Sele the "Add a ne instrument" button if you instrument not on the list Lastly, sele whether tł MS or MS/M data has bee centroided. Click tł "Next" butto When only or instrument selected. PEAKS star loading data.

1. Project Properties	Which instrument(s) was(were) used to p	roduce these data?	
<ol> <li>Project Properties</li> <li>Sample Properties</li> <li>Instrument Details</li> <li></li> </ol>	Thermo Scientific	Instrument Details: LCQ Ion Source: MS Scan: Frag - type: MSn: Default Parent mass tol.: Default fragment tol.: MS centroid: MSMS centroid:	ESI(nano-spray) 3D Ion Trap CID, CAD, IRMPD (y and b ion 3D Ion Trap
	LTQ FT Ultra Hybrid FT-FT (p LTQ FT Ultra Hybrid FT-Trap Add a new instrument	Back Next >>	Cancel Help

7) If more than two instruments are selected, the "Instruments and Files" window will open next. The top of the window will contain a list of all files selected for the project. The lower part of the window contains the instruments selected in the "Instrument Details" window.

Steps	Instruments and Files				
<ol> <li>Project Properties</li> <li>Sample Properties</li> <li>Instrument Details</li> <li>Instruments and Files</li> </ol>	This list contains all the files produced them. Name on\data\jain\297.RAW on\data\jain\274.RAW on\data\jain\276.RAW	Size 84,327 KB 91,301 KB	ect. But PEAKS doesn't kno Date Modified 03/03/2006 06:54:00 AM 03/02/2006 11:50:00 AM 03/02/2006 10:05:00 AM	Туре	rav rav rav
	To associate a file with an in list below. Use CTRL + Click LTQ FT Ultra Hybrid FT LCQ Fleet Ion Trap LCQ Fleet Ion Trap (pq	k or SHIFT + Click to select -FT (etd)		o the instrument in th	ъ
	Reset	)			

8) In order to indicate which instrument was used to generate each file, drag the file from the list above and drop it onto the instrument in the list below. In order to drag and drop multiple files at once, use Ctrl + Click or SHIFT + Click. Use the reset button to return the files to the list at the top of the window if you make an error.

eps	Instruments an	nd Files					
1. Project Properties 2. Sample Properties	This list contains a produced them.	This list contains all the files you selected for this project. But PEAKS doesn't know which instrument produced them.					
10 (1996) 1997	Name	Size	Date Modified	Туре			
Instrument Details							
	LTQ FT Ultra	RL + Click or SHIFT + Click Hybrid FT-FT (etd) lata\iain\266.RAW 1 Trap lata\iain\297.RAW	it from the list above and drop i to select several files.	t onto the instrument in th			

9) Click on the finish button and the file will begin loading.

## 4.5 Open a Project

Go to "File" and select "Open Project" or "Open Recent Project" in to access stored projects. You can also select "Close project" in the close a project that is open.

📥 P	EAKS			
File	Tools	Window	Help	
	o New	Project		Ctrl+Shift+N
	👌 New	File		Alt+Shift+N
	📄 Ope	n Project		Ctrl+Alt+O
	📄 Ope	n Recent P	roject	•
	👩 Clos	e Recent P	roject	•
Ę	Save	e As		Ctrl+Shift+S
8	🔊 Prin	t		
	🕈 Exp	ort		•
E	3 Exit			Ctrl+Q

#### 4.6 Changing the Location of Saved Projects

Projects are saved in the location that is listed in your "Preferences" window. To modify your preferences, select the "Preferences" toolbar icon  $\checkmark$  or select "Preferences" from the "Window" menu. Select "General" on the left hand side of the window. The default "Output Directory" and "Project Folder" locations are listed in the "Default Output Directory" panel. Please note that the defaults seen here may differ from your default locations depending where you downloaded PEAKS. Click on the "Browse" buttons to change either of these locations.

Default Output Directory	
Output Directory	Browse
C:\derbyServer	
Project folder	Browse
C:\derbyServer\serverDB	

You can also change the location of you projects on a project by project basis by selecting a new "Project Location" when setting up a database as seen below:

📐 New Project			
Steps	Project Properties		
1. Project Properties	Project Name:	Project 1	]
2	Project Location:	C:\Peaks Studio 5.0\.\derbyServer\serverDB	Browse

## 4.7 Orienting Yourself

#### **Project View Panel**

This frame appears in the upper left hand corner, displays the organization of a particular project (if applicable) or simply of a data file. Use the '+' and '-' boxes to expand

and collapse the project in order to access the data file that you want to analyze. Make sure the data file to be analyzed is selected.

#### **Properties Panel**

PEAKS reads and tracks information about the experiment for use in the analysis and for future reference. Once the data file has loaded, click on the properties tab in the bottom left hand corner. If any information cannot be found in the file, PEAKS will prompt you to enter this information.

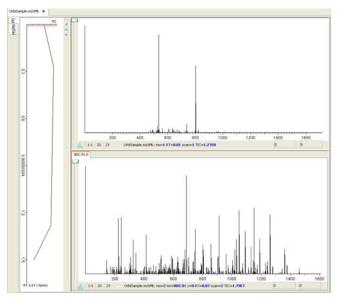


Selection Details: 9Prot	einMix_FT.RAW
Total MSI Spectra	1283
Total MS/MS Spectra	3712
Ion Source	ESI(nano-spray)
Fragmentation Mode	CID, CAD, IRMPD (y
MS Scan Mode	FT-ICR/Orbitrap
MS/MS Scan Mode	Linear Ion Trap
MS Scan Centroid	false
MS/MS Scan Centroid	false

#### Raw Spectrum View

Opening the raw file in PEAKS will display the following graphs in the "Main Processing Window".

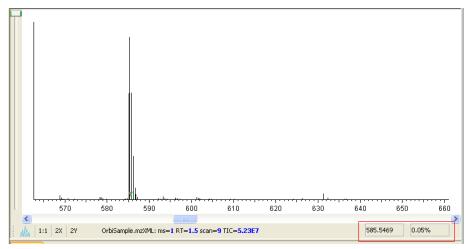
The "MS" tab is selected by default and represents the precursor scan. On the left hand side of the screen is the total ion current (TIC). Depending on how the file was generated there may be simply a list of spectra and not a TIC graph. The retention time is plotted against the vertical axis. Clicking on the TIC graph will move the red line and display the ms spectra to the right of the TIC graph that corresponds to the selected retention time. Alternatively, use the up and down arrows, found on the keyboard, to move



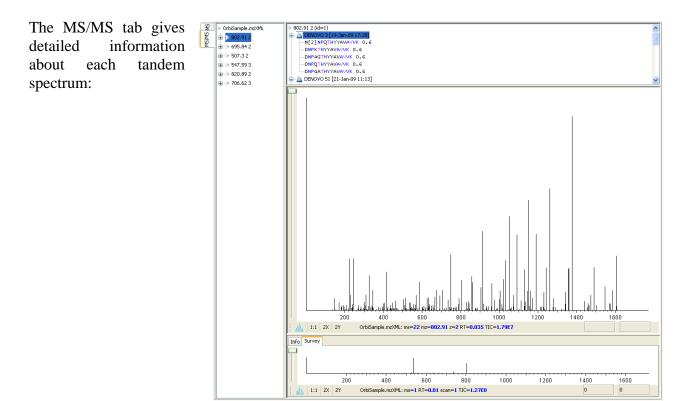
through the TIC. If the ms2 scans is available, it will be displayed below the corresponding ms scan.

To zoom either on the X or Y axes, select the "2X" or "2Y" buttons, respectively. To scroll in even more, click the button on the left of your mouse and drag the arrow to the side. To increase the intensity of the peaks, use slide the scroll bar on the left hand side, up and down. Selecting the "1:1" button will bring you back to the original image where the entire spectrum is visible.

Scrolling over the spectrum will display the m/z ratio and the height/intensity (as a percentage of 100) of the particular peak under the spectrum view on the right hand side (see the box highlighted in red below).



You can use the profile and peak in buttons to switch the spectrum view from profile mode to peak mode and vice versa.



Each of the spectra in the data file will be listed in the left most panel under the name of the data file. Clicking on one of the spectra will display the results that have been generated for that spectrum in the top right hand panel (as seen in the example above). Before any results files have been generated, the top panel will look like this:

Ω	▶ OrbiSample.mzXML	no result (no result)
SM/SM	802.91 0	
ĽΣ	695.84 0	
	507.3 0	
	547.590	
		-

ĺ	Info Survey
	Selected MS/MS [OrbiSample.mzXML, 802.91 2] Retention Time: 0.035 TIC: 1.79E7Number of Peaks: 222 Fragmentation Type: Unavailable
	Number of Results: 7 DENOVO 3 [19-Jan-09 17:28] has 5 matches DENOVO 51 [21-Jan-09 11:13] has 1 matches PEAK5 1 [19-Jan-09 17:22] has 1 matches PEAK5 2 [19-Jan-09 17:26] has 1 matches PEAK5 3 [19-Jan-09 17:29] has 1 matches DENOVO 1 [19-Jan-09 17:21] has 5 matches DENOVO 2 [19-Jan-09 17:25] has 5 matches

More information can be found about the spectra under the "Info" tab. You will find information about the retention time, where to find the spectra on the TIC graph, the number of peaks and the fragmentation type (if available). You will also find an overview of the results that were found for that spectrum in the results files.

The largest panel displays the MS/MS and below you will find the corresponding MS spectra under the "Survey" tab. Information about navigating through the MS and MS/MS spectra can be found above in the section describing the "MS" tab.

# Chapter 5

# 5. Data refinement

Since mass spectrometry data often contains noise and redundant data, it makes sense to filter the data before analysis. This will increase the quality of the results, while saving time spent on database searching and *de novo* sequencing. MS/MS spectra that are mostly noise will be removed from the data.

When PEAKS is connected to a PEAKS Online server, you will also save time by uploading smaller, preprocessed data. Data refinement can be done locally, before uploading to the server.

#### 5.1 Run Data Refine

To begin the refinement of data from a whole MS/MS run:

- 1) In the "Project View Frame", select the data file(s) containing the data that you wish to refine.
- 2) Click the Data Refine toolbar icon  $\propto$ 
  - Or

Select "Data Refine" from the "Tools" menu.

ools	Data Refine					
√. Data Refine	Merge Options					
	Merge scans of the same peptide:	Merge scans of the same peptide:			🔿 no	
	Retention time window: (for raw file:	s only)	1		min.	
	m/z tolerance:	m/z tolerance: 0.01				
	Charge Options					
	Correct precursor charges:			() yes	🔿 no	
	Minimum charge: 1	Maximum o	harge: 4	A CONTRACTOR OF		
	Filter Options					
	Filter MS/MS scans:			💽 yes	🔿 no	
	Precursor mass between	350	and	6000	224	da
	Retention time between	10	and	200		min
	Quality value greater than	0.65	suggest 0	.65		
	Preprocess Options					
	Preprocess MS/MS scans:	0	io, already do	ne 💿 yes	🔿 no	
		ОК	Cancel			Help

The Data refinement options window will appear:

3) Choose the data refinement tools you wish to use by clicking the "yes" radio button next to each one. See the information below to help you decide on proper refinement parameters.

#### 5.2 Data Refinement Parameters

#### Merging Scans

In DDA mode, a mass spectrometer will often produce several tandem ms (MS/MS) scans of the same peptide. To increase the intensity of real signal peaks within these scans and to reduce the size of the whole data set, it makes sense to merge MS/MS scans of the same peptide together. To avoid improper merging (of MS/MS scans of different peptides) we make sure that the measured parent ion masses of these peptides are very close and that they have similar retention times in the LC column. The units here are m/z values in Daltons. For retention time, we use whatever units are recorded in the data file (usually minutes or seconds).

#### Precursor Charge Correction

Since a mass spectrometer measures mass-to-charge ratios, we must know the charge on a peptide before we can determine its mass. The standard method of finding the charge is to look at the spacing of the isotope ladder in the survey scan. However many Ion-Trap instruments do not have enough resolution for this. So PEAKS will look at the MS/MS data to determine if it's charge 1+, 2+ or 3+. For data where the survey scan is available, PEAKS will examine the precursor ion's isotope distribution to confirm or correct the charge assignment. Type in the boxes to set a range of charges. Only spectra that fit in this range will be considered for analysis.

#### Filtering MS/MS Scans

Scans of contaminants and electrical noise should not be included in analysis. Removing them from the data set will save time, and reduce the risk of random matches to the database. PEAKS offers an effective tool for removing these low quality MS/MS scans. Type in the boxes to set ranges of retention time and m/z ratio. Only peaks between these values will be considered for analysis. Additionally, PEAKS examines the MS/MS spectrum to determine its quality. The quality filter is based on four characteristics: signal to noise ratio over MS/MS, number of peaks after pre-processing, sum of all peak intensities and length of the longest simple sequence tag that can be generated. You can choose a threshold of quality score (a value from 0 to 1) for accepting a scan. We recommend a quality filter of 0.65. Set to 0.01 to disable quality filtering.

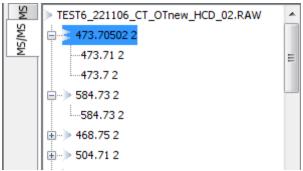
#### **Preprocessing MS/MS Scans**

This section deals with deconvolution (de-isotoping), centroiding and noise filtering within the MS/MS data. Preprocessing can save hard disk space or upload time. But make sure to have the original data available in case you need to refer to it later.

To see how your data is changed after data refinement, refer to the data properties window.

#### 5.3 Data Preprocessing Results

To view the result of data pre-processing, click on the MS/MS tab on the spectrum view. In following example, the spectrum (m/z = 473.70502) results from raw spectra (m/z = 473.71 and m/z = 473.7).



# Chapter 6

# 6. De novo Sequencing

# 6.1 Setting up Auto De novo Sequencing Parameters

1) In the "*Project View Frame*", select the data file(s) or project containing the spectra that you wish to sequence by Auto *de novo*.

2) Click the Automatic *de novo* toolbar icon Or

Select "Auto *de novo*" from the "Tools" menu.

appear:	Mass Options		
appear.	Parent Mass Error Tolerance: Fragment Mass Error Tolerance:	0.15	da 🗸
	Enzyme Options		
	Enzyme: Try	/psin	New Enzyme
	Digest Rule:	{P}	Delete Enzyme
	Find peptides that satisfy at both	ends	Advanced
	PTM Options		
	Name	Mono Residu	Fixed Modification
	Acetylation (N-term)	1 · · · · · · · · · · · · · · · · · · ·	Cxidation
	Acetylation (K)	42.010567 [K],	
	Amidation	-0.984016 [X]@C,	
	Applied Biosystems original ICAT(TM)		C Variable Modification
	Applied Biosystems original ICAT(TM)		Iodoacetic acid derivative
	Applied Biosystems cleavable ICAT(T/ Applied Biosystems cleavable ICAT(T/		
	Applied biosystems cleavable ICAT(T	M) heavy 236.15718 [C],	<u>×</u>
	Show unimod	New PTM	Max variable PTM per peptide: 3
	General Options		
	Preprocess this data 'on the fly'	(deconvolute, filter noise, centroid)	Report up to (# peptides): 5 💲

3) To change any of the following parameters, now is the time:

#### Mass Options

*Parent mass error tolerance:* Determine how much random and systematic experimental error on the parent/precursor ion PEAKS will allow for in its analysis. As you have previously selected your instrument, PEAKS will provide the suggested error tolerances. Type a tolerance in the textbox and choose units from the dropdown list. Using PPM allows for larger errors at larger m/z values. PEAKS will be very stringent concerning this value, so new PEAKS users should try setting this a little higher than past experience may suggest, if sensitivity is a concern.

*Fragment mass error tolerance:* Determine how much random and systematic experimental error on the fragment/daughter ion PEAKS will allow for in its analysis. As an instrument has previously been selected, PEAKS will provide suggested error tolerances. Type a tolerance in the textbox. Again, new PEAKS users should try setting this a little higher than past experience may suggest.

#### Enzyme Options

*Enzyme:* Tell PEAKS what type of enzyme was used to digest the sample. Choose from a dropdown list of enzymes, or if your enzyme is not in the list, click the "New Enzyme" button. You can then input the name of the new enzyme.

*Digest Rules:* Enter the amino acid that is found at the end of the peptide. Put set brackets {} around a residue to denote any amino acids except for those that are within the brackets. Select the "Advanced" button if your digest rules are more complicated. Select the radio box "Select peptides that satisfy at both ends" if you require that your peptide was cut by the enzyme you chose at both ends.

#### **PTM Options**

*Selecting fixed and variable PTMs:* The "PTM Options" list tells PEAKS what types of posttranslational modifications to include in its analysis. To view additional modifications, select the "Show unimod" box. If a desired PTM does not appear on the list or is different than what is listed, select the "New PTM" button and the "PTM Editing" window will open. Fill in the information pertaining to your PTM. To select a PTM as Fixed or Variable, drag the PTM into the Fixed Modification or Variable Modification box. If you drag over an incorrect PTM, simply drag it back to the "PTM Options" list.

*Max variable PTM per peptide*: To reduce uncertainty, limit PEAKS' *de novo* sequencing 'vocabulary' by restricting the number of variable PTM found on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).

#### General Options

*Report up to (# peptides)*: Set how many peptide sequences PEAKS will report from its *de novo* sequencing analysis.

*Preprocess your data "on the fly" before auto de novo*: PEAKS has its own built-in preprocessor for removing noise, centroiding and deconvolution. Check this box to turn preprocessing on. BSI highly recommends using PEAKS to preprocess all data, as opposed to using instrument vendor software, if the data is to be used by PEAKS. PEAKS preprocessor should not be used on data that has already been pre-processed as this will have adverse effects on the results (unless it is ion-trap data).

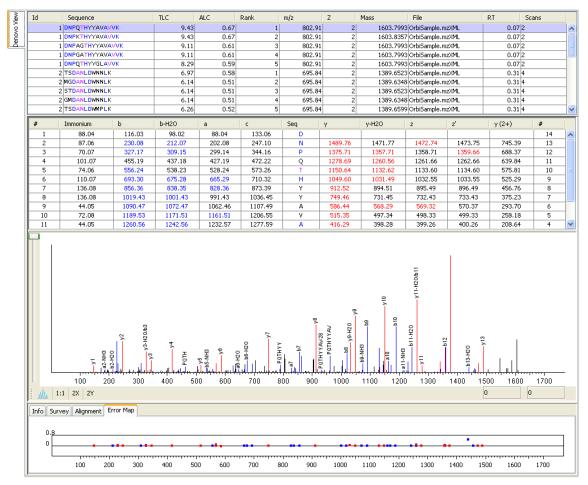
Note: If you have already pre-processed your data in the data refinement step, you do not need to do this again.

4. After setting parameters, you can save them for future use. Click the "Save Parameters" button at the top of the window, and choose a name for future reference when prompted. Any parameters that are saved will be available in the drop-down list at the top of the window. To see what's inside, select one and the parameters boxes will be populated.

5. Press the "OK" button to initiate *de novo* sequencing.

#### 6.2 De novo Sequencing Results

Once *de novo* sequencing is finished, the following window will open:



#### Peptide Candidates Frame

PEAKS displays the peptide sequence candidates at the top of the screen in the "Peptide Candidates Frame". You can sort the results by clicking on any of the titles of the columns. For example, to sort the peptide sequence candidates by ID click on "ID". Note that all of the peptides that have the same ID have the same mass, charge, retention time and quality score. See page 43 for more information on how the quality score is generated.

A unique identifier for the MS/MS ID spectrum. This differs from a scan number since we may have merged several scans together. The sequence of the peptide (including Sequence modifications if present) as determined by de novo sequencing. TLC Total local confidence (the confidence that we have in the peptide sequence). It is calculated by adding the positional confidence for each amino acid in the peptide sequence. ALC Average local confidence (the confidence that we have in the peptide sequence). It is calculated by adding the positional confidence for each amino acid in the peptide sequence and dividing by the total number of amino acids. The sequences for a particular spectrum Rank (ID) as sorted by score (TLC). The measured mass/charge value, in m/zDaltons, for the peptide. The calculated charge value for the peptide. Ζ Calculated using the measured m/z and Mass calculated z, we use this as the experimental mass of the peptide. File The name of the file. RT Retention time (elution time) for the peptide as recorded in the scan header. The scan number. Scan A value from 0 to 1 estimated from the Quality spectrum to refer to spectrum quality. Attributes like signal to noise, total intensity, and spectrum tagging are used. Mode that the scan step was performed in. Scan Mode Mode that the fragmentation step was Frag. Mode performed in.

The following table describes the contents of the columns in the "Peptide Candidates Frame":

The columns themselves are customizable. Right click anywhere in the report and choose Toggle Column from the pop-up menu. The sub-menu that appears shows a checkmark in each of the columns that are currently showing. Click any one of them to show or hide a column. These settings will apply to all your reports.

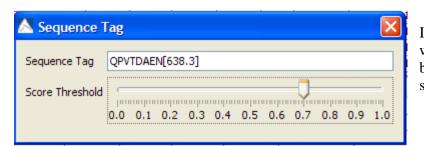
#### **Confidence Scores**

Next to the proposed sequence candidates, the auto *de novo* "Total Local Confidence" (TLC) and "Average Local Confidence" (ALC) confidence scores are shown. The confidence scores for each amino acid (that is, confidence that the correct residue in each position has been identified) are represented by color coding. **Red** represents a very high confidence (greater than 90%), **purple** represents a high confidence (80 to 90%) **blue** represents a medium confidence (60 to 80%) and **black** represents a low confidence (less than 60%). For more detailed positional confidence, place the cursor over the sequence of interest and right click "Show Positional Confidence". A "Position Confidence Table" will appear, showing the confidence that each amino acid/pair of amino acids are correct.

#### **Sequence Tags**

Right click on a peptide in the "Peptide Candidates Frame" and select "Show Sequence Tag". If the score threshold is set at 0.0, all of the amino acids in the peptide sequence will be displayed.

🔼 Sequence 1	Tag	×
Sequence Tag	QPVTDAENCHALR	
Score Threshold	I	_
	0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9	1.0



Increasing the "Score Threshold" will display a mass in square brackets if the amino acids do not satisfy the score threshold.

#### **Modifications**

Consider the following sequence: DW[1]C[1]SFTDAENVQALAR

The number 1 in square brackets refers to where a modification may occur. If you forget what modifications you selected before running *de novo*, click to the "Properties" tab.

The fixed modification is set to [1]58.005478@[CKW]. In the sequence above, the modification has been made to the W as well as the C. The colors assigned to the [1] follow the same confidence scores as the amino acids themselves.

Refer to the above section on "Confidence Scores" for more information on color coding.

#### Denovo details: Test 6

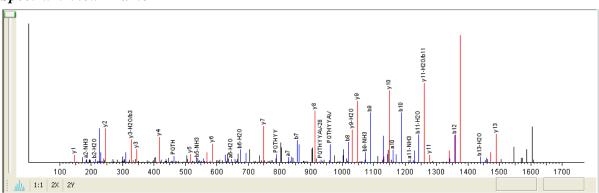
Parent Mass Error Tolerance	0.1 da
Fragment Mass Error Toler	0.6 da
Enzyme	Trypsin
Semi (enzyme)	false
Fixed Modification	[1]58.005478@[CKW],
Max variable PTM per peptide	2
Report # peptides	5
Preprocess data	false

#### Ion Table Frame

#	Immonium	Ь	Ь-Н2О	а	c	Seq	у	y-H2O	z	z'	y (2+)	#
1	88.04	116.03	98.02	88.04	133.06	D	1	1	1	1		14
2	87.06	230.08	212.07	202.08	247.10	N	1489.76	1471.77	1472.74	1473.75	745.39	13
3	70.07	327.17	309.15	299.14	344.16	P	1375.71	1357.71	1358.71	1359.66	688.37	12
4	101.07	455.19	437.18	427.19	472.22	Q	1278.69	1260.56	1261.66	1262.66	639.84	11
5	74.06	556.24	538.23	528.24	573.26	T	1150.64	1132.62	1133.60	1134.60	575.81	10
6	110.07	693.30	675.28	665.29	710.32	н	1049.60	1031.49	1032.55	1033.55	525.29	9
7	136.08	856.36	838.35	828.36	873.39	Y	912.52	894.51	895.49	896.49	456.76	8
8	136.08	1019.43	1001.43	991.43	1036.45	Y	749.46	731.45	732.43	733.43	375.23	7
9	44.05	1090.47	1072.47	1062.46	1107.49	A	586.44	568.29	569.32	570.37	293.70	6
10	72.08	1189.53	1171.51	1161.51	1206.55	V	515.35	497.34	498.33	499.33	258.18	5
11	44.05	1260.56	1242.56	1232.57	1277.59	A	416.29	398.28	399.26	400.26	208.64	4

The "Ion Table" shows the proposed ions with their corresponding masses. To add additional ions to the ion table, see the instructions on page 95.

If an ion is found in the corresponding spectrum, it must first pass two criteria before being displayed in a specific color (blue for N-terminal ions and red for C-terminal ions). It must be found within the mass error tolerance chosen by the user and must have an intensity of greater than 2% of the ion with the greatest intensity.



#### Spectrum View Frame

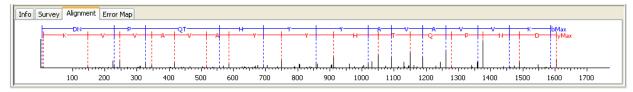
The "Spectrum View Frame" is found below the "Ion Table" and displays a graphical representation of the spectrum. The peptide that corresponds to the spectrum in the "Spectrum View Frame" is displayed in the "Input Sequence" box. Use the drop down to select other peptides that have the same ID.

Scrolling over the spectrum will display a "tooltip" in the new window that will display the m/z ratio and the height/ intensity (as a percentage of 100) of that particular peak. Both the m/z ratio and the height of the peak can also found under the spectrum view on the right hand side.

To zoom either on the X or Y axes, select the "Zoom X" or "Zoom Y" buttons, respectively and then use the wheel on your mouse to move around the graph. Selecting the "1:1" button will restore settings to view the entire spectrum on the screen.

You can use the profile and peak to buttons to switch the spectrum view from profile mode to peak mode and vice versa. The scrollbar on the left acts to increase and decrease the intensity of the peaks, where the scrollbar on the right acts to zoom in to display the monoisotopic peaks.

#### Spectrum Alignment Frame



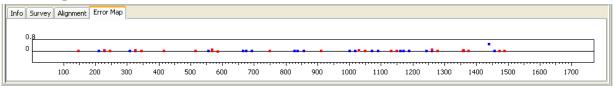
Clicking on the "Alignment" tab will display the "Spectrum Alignment Frame". This frame always shows the whole spectrum and is used as a tool to help us navigate the spectrum view frame. A blue bar along the horizontal axis of the alignment view indicates the range of the spectrum view in the Spectrum View Frame. This frame will show you how the proposed ions align with the spectrum. By default, the Spectrum Alignment Frame displays b-ions and y-ions. The b ions are shown right to left in blue, while the y ions are shown left to right in red.

#### Survey Scan

Info	Survey	Alignment	Error M	lap														
	1					l			I									
l		100	200	300	400	<del>امبرا</del> 500		,,	<b> </b> 800	,, 900	1000	1100	1200	1300	1400	1500	1600	1700
1	1:1	2X 2Y	Orb	oiSample.m	zXML: ms=	1 RT=0.01	scan= <b>1</b> T	IC= <b>1.27E</b> 8										

Clicking on the "Survey" tab will display the corresponding precursor ion spectrum. The buttons that appear in this section are the same as those that are explained above in the "Spectrum View Frame" section.

#### Error Map



Click on the "Error Map" tab. The m/z ratio is displayed on the y axis and the error is listed on the x axis in Daltons. The "Error Plot" displays the confidence that is assigned to each ion. The most confident results lie on the centerline. Clicking a cell or column in the Ion Table highlights the corresponding points on the error plot and corresponding peaks on the spectrum.

# Chapter

# 7. Database Search

# 7.1 Setting up Protein Identification Parameters

1) In the "*Project View Frame*", select the data file(s) or project containing the spectra that you wish to identify using database search.

2) Click the Protein Identification toolbar icon  $\mathbf{M}$ 

Or Select "PEAKS Protein ID" from the "Tools" menu.

The Protein	Database Search		Save Parameter	test	6	~
Identification Parameters dialogue	Mass Options Parent Mass Error Tolerance: Fragment Mass Error Tolerance:	0.1 da 💌 0.6 da 💌	Precursor Mass Search Tyj	his data 'on the fly' avages: 3 🔷		
window will appear:	Enzyme Options Enzyme: Digest Rule: Image: Find peptides that satisfythe PTM Options	Trypsin RK above rule at both end	is			New Enzyme Delete Enzyme Advanced
3) To change any of the		1567 [AC 1567 [K] 016 [X] 25 [C] 752 [C] 2698 [C] 5718 [C]	@C,	Variable Modification		
protein identification search parameters, now is the time.	Paste fasta sequences	lect Database: Swiss I	Prot Database Edit Dat Set Ta	abase	species	
	Advanced Options PEAKS uses a hybrid search tech I have already run de novo, Run de novo using different Run de novo using the same Validation - decoy search	don't run it again parameters than the a	bove	the search		V

#### Mass Options

*Parent mass error tolerance*: Determine how much random and systematic experimental error on the parent/precursor ion PEAKS will allow for in its analysis. Type a tolerance in the textbox and choose units from the dropdown list. Using PPM allows for larger errors at larger m/z values. PEAKS will be very stringent concerning this value, so new PEAKS users should try setting this a little higher than past experience may suggest, if sensitivity is a concern.

*Fragment mass error tolerance*: Determine how much random and systematic experimental error on the fragment/daughter ion PEAKS will allow for in its analysis. Type a tolerance in the textbox. Again, new PEAKS users should try setting this a little higher than past experience may suggest.

*Precursor mass search type*: If the precursor mass is monoisotopic value, check monoisotopic. Check average, otherwise.

#### **Enzyme Options**

*Enzyme*: Indicate which type of enzyme was used to digest the sample. Choose from a dropdown list of enzymes. Note that you cannot delete or change the details of a built-in enzyme and therefore the "Delete enzyme" button and the "Digest Rules" panel will be grayed out. If your enzyme (or combination of enzymes) is not in the list, click the "New Enzymes" button. You will then be able to enter a name for your enzyme, digest rules (see below) and select if you would like to find proteins that satisfy the rules at both ends. This option is grayed out for built-in enzymes.

*Digest Rules*: This is how you specify where your enzyme will cleave the protein between two amino acids to create peptides. The letter X denotes 'any amino acid in this position', while {set brackets} indicate any amino acid except the one in the brackets. Clicking on the "Advanced" button will open a new window which will allow you to be more specific with your digest rules.

igest Rules	Cleavage Site	
residues at the end of a peptide		start of a new peptide
And/Orresidues at the end of a peptide		start of a new peptide
And/Orresidues at the end of a peptide		start of a new peptide
And/Orresidues at the end of a peptide		start of a new peptide
Find peptides that satisfy the above rules at bo	th ends	

#### General Options

*Max missed cleavages*: determine the most missed cleavages to allow, internal to the peptide, in a *de novo* sequence. For instance, setting this to 2, and Trypsin as the enzyme, then PEAKS will return *de novo* sequences with up to 2 R's or K's internally.

*Preprocess before auto de novo*: PEAKS has its own built-in preprocessor for removing noise, centroiding and peak charge recognition from MS/MS data. Check this box to turn preprocessing on.

#### PTM Options

*PTM options*: This list tells PEAKS what kind of post-translational modifications to include in its analysis. Drag the desired PTM into either the "Fixed Modification" or "Variable Modification" box. If the desired PTM is not in the list, first check the "Show Unimod" box to show additional PTMs. To create a new PTM click on the "New PTM" button. The following window will appear:

A PTM Editing	
PTM name: Mass (Monoisotopic): Neutral loss mass (Monoisotopic): Residues that can be modified: Formula: Rule:	Anywhere
	Ok Cancel Help

Fill in the following information:

Name: this name will appear in the PTM list for future use after it is saved.

*Monoisotopic mass*: the mass that the residue gains or loses as a result of the PTM. Enter this value numerically.

*Neutral loss mass*: the mass that the modified residue loses as a result of fragmentation. Ex. 28 would signify a loss of 28 Daltons. This is optional.

*Chemical formula*: the chemical formula of the PTM. This should correspond to the mass listed above. This is optional.

*Residues that can be modified*: Enter residues that can be modified anywhere, residues that can only be modified if they are at the N-or C-terminus or in the middle only.

*Rule*: Enter comments for reference. This is optional.

Please note that you can also configure your PTMs in the "Configuration" panel. See page 98 for more information.

*Max variable PTM per peptide*: To reduce uncertainty, limit PEAKS' *de novo* sequencing 'vocabulary', by restricting the number of variable PTM found on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).

#### Database Options

*Database to search*: Select from this dropdown list, one of the FASTA databases configured in PEAKS. To edit an already existing database, click on the "Edit Database" button. If the desired database is not in this list, click the "New Database" button. Note that you can also set up a new database in the "Database Configuration" window. The configuration window is the only place that that you can delete databases that you have created. For more information on setting up new databases see page 99.

*Taxonomy selection*: This list displays the taxa you have chosen for your search. If the database selected has taxon information available, you can click on the "Set taxa" button. Otherwise, the whole database will be searched. The selections correspond to established hierarchy -- i.e. selecting 'Mammalia' will search all of 'horse, cow, rat, mouse, human, etc.

*Paste FASTA sequences*: If you already know the sequence of the protein(s) you are looking for, select "Paste fasta sequences" and paste the sequence in the space provided in fasta format. Alternatively, if you want search the same sequence regularly, it is recommended to simply create a small text file and configure it as a database for PEAKS.

#### Advanced Options

PEAKS needs to have some *de novo* sequences before database searching since PEAKS uses sequence tags to perform database searching. As such the option of doing *de novo* prior to protein ID is presented here. In most cases, the same values for instrument, error, enzyme and PTM can be used in *de novo* and in protein ID, but you have the option of using one of your saved *de novo* parameter sets for the *de novo* portion. Select one from the drop down list.

4) After setting up parameters, we can save them for future use. Click the "Save Parameters" button, and choose a name for future reference when prompted.

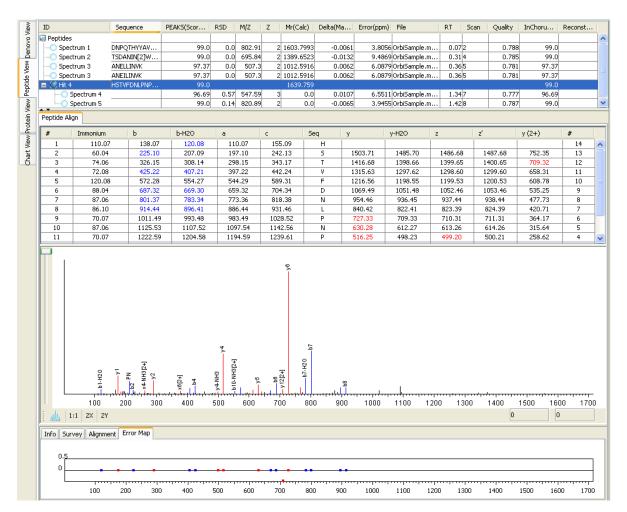
Any parameters that you save will be available in the drop-down list at the top of the window. To see what's inside, just select one, and the parameters boxes will be populated.

5) Press the "OK" button. If you have already performed *de novo* sequencing, the database search will commence automatically. If you have not previously performed *de novo* sequencing, the auto *de novo* process will appear first in the task queue. Once *de novo* sequencing is finished the database search will begin.

# 7.2 Protein Identification Results

#### Peptide View

Once PEAKS is finished searching the database, the "Peptide View" window will open by default:



The "Peptide View" window summarizes the results for each MS/MS spectrum. All peptides that match to each spectrum are displayed. By default the spectra are listed by ID in the "ID" column with the corresponding peptide sequence in the "Sequence column" beside. In certain cases, one peptide can correspond to more than one spectrum. These spectra are then listed in the "ID" column under a heading entitled "Hit". Click on "+" to expand the view to see all of the spectra that can be matched by the same peptide.

The table below describes the contents of the columns in the "Peptide View Window":

ID	A unique identifier for the MS/MS
	spectrum. This differs from a scan number
	since we may have merged several scans
	together.

Sequence	The amino acid sequence of the peptide.
Sequence	PTMs are listed in [square brackets].
C	
Score	PEAKS' probability score.
m/z	The measured mass/charge value, in
	Daltons, for the peptide.
Ζ	The calculated charge value for the peptide
Mr (Calc)	The sum of the theoretical mass of the
	residues that form the identified peptide
	sequence from the database.
Delta (Mass)	The difference between Mr(Calc) and
	Mass, in Daltons.
Error (ppm)	The difference between Mr(Calc) and
	Mass, ppm.
File	The name of the file.
RT	Retention time (elution time) for the
	peptide as recorded in the scan header.
Scan	The scan number.
Quality	A value from 0 to 1 estimated from the
	spectrum to refer to spectrum quality.
	Attributes like signal to noise, total
	intensity, and spectrum tagging are used.
Scan Mode	Mode that the scan step was performed in.
	* *
Frag. Mode	Mode that the fragmentation step was
	performed in.

The columns themselves can be customized. Right click anywhere in the report and choose Toggle Column from the pop-up menu. The sub-menu that appears shows a checkmark in each of the columns that are currently showing. Click any one of them to show or hide a column. These settings will apply to all your reports.

#### Peptide Alignment

Click on the "Peptide Align" window. This will look very similar to the *de novo* results window. You will see the "Ion Table" which shows the proposed ions with their corresponding masses. To the right of the "Ion Table" is the "Error Plot" which displays the confidence that is assigned to each ion. The most confident results lie on the centerline. Clicking a cell or column in the Ion Table highlights the corresponding points on the error plot and corresponding peaks on the spectrum.

Underneath the "Ion Table is the "Spectrum View Frame" which displays a graphical representation of the spectrum. The peptide that corresponds to the spectrum in the "Spectrum View Frame" is displayed in the "Input Sequence" box. Note that this is a drop down menu so that you can select other peptides that have the same ID (if applicable). Scrolling over the spectrum will display a "tooltip" that will display the m/z ratio and the height/ intensity (as a percentage of 100) of that particular peak. Both the m/z ratio and the height of the peak can also found under the spectrum view on the right hand side.

To zoom either on the X or Y axes, select the "Zoom X" or "Zoom Y" buttons, respectively and then use the wheel on your mouse to move around the graph. Select the "Slide X" button and then use the wheel on your mouse to move around the graph. You must ensure that you are sufficiently zoomed in on the X axis to use the "Slide X" button. Selecting the "1:1" button will bring you back to the original image where you can see the entire spectrum on the screen.

You can use the profile and peak buttons to switch the spectrum view from profile mode to peak mode and vice versa. The scrollbar on the left acts to increase and decrease the intensity of the peaks, where the scrollbar on the right acts to zoom in to display the monoisotopic peaks.

Finally at the bottom of the screen is the "Spectrum Alignment Frame" which is used as a tool to navigate the "Spectrum View" frame. A blue bar along the horizontal axis of the alignment view indicates the range of the spectrum view in the "Spectrum View Frame". This frame will show you how the proposed ions align with the spectrum. By default, the "Spectrum Alignment Frame" displays b-ion and y-ion. The b ions are shown right to left in blue, while the y ions are shown left to right in red.

#### **Peptide Details**

Click on the "Peptide Details" tab. The following window will appear:



At the top of the "Peptide Details" frame is the accession number of the protein that corresponds to the peptide that you chose in the "Peptide View" window. If more than one protein matches a single peptide, you will be able to select these additional proteins using the dropdown menu.

Below this you will see a simple alignment between the original *de novo* sequence for this spectrum (if available), the peptide found in the database and the reconstructed sequence. Letters on a green background, and with vertical bars, indicate agreement. Color codes on the *de novo* sequence letters still indicate positional confidence.

Finally at the bottom of the window you will see the sequence of the selected protein and in blue you will see where the selected peptide matches the protein. The darker the blue, the more confident the match is. The matched peptides will be shown in red if you have performed a SPIDER search which is discussed in the next section.

#### **Protein View**

Click on the "Protein View" tab. The following window will appear:

Accession	ID		Mass		Pisplay		(Score %) Cove	rage(%)	Query matched			
DB Search				c 400 0 4			00.45	10.11				<b>N</b> 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
🖬 🛄 P00722 BGA		2		6482.8			99.15	10.16	1			Beta-galactosic
Q29443 TRF				7753.2			99.15 99.1	15.48	1			Serotransferrin
0 P02769 ALB		3		293.55				14.99	1			Serum albumin
A7ZUE0 GLP		4		30.816		_	98.81	7.97		4		Glycerol kinase
P49064 ALB		14		659.56			98.52	7.57		5		Serum albumin
Q28522 ALB		27		881.09			91.41	5.67		3		Serum albumin
P00698 LYS		29		38.638			83.72	17.69		2 2		Lysozyme C pr
Q4UZR8 GLF		31 49		5229.5 1 23.152	-		83.72	4.41		2		Glycerol kinase
P00330 ADH 0 007704 AUD							83.69	5.17		2 3		Alcohol dehydr
		52 60		692.59			70.87 60.7	4.11 7.47		5 1		Serum albumin
Q9Y707 AC1				63.453	-	-				2		Actin-2 - Suillus
Q1JP56 OPM		63		489.82	_	-	60.7 60.7	4.8 11.63		1		Melanopsin-like
P00706 LYS		64 79		6881.0	_		60.7	3.85		2		Lysozyme C-3
Q48K59 1A1		79 81		57.504			60.7	2.23		3		1-aminocyclopr Formatetetra
		85		.82.424			60.7	5.45		5 1	H	Alpha-1-acid gl
Q352R3[A17 B-O Q6DKE1[CY0		88		95.467			60.7	9,52		1	H	Cytochrome c,
■ O Q00KE1[CYC		91		82.467			60.7	9.52		1	H	Cytochrome c -
Q03131  ER1	-	124		02.467			60.7	9.52		2	H	Erythronolide s
■ O Q92BH6 GLF		124		435.76			60.7	2.01		1	H	Glycerol kinase
	-	120					00.7	2.01		1		
NCBI BLAST sear Link to retrieve e		722 BGAL_EC			trez:							
NCBI BLAST sear	ch of 1900	1722 BGAL_EC	COL) equence fro	m NCBI En	trez:		Description					
NCBI BLAST sean Link to retrieve e	ch of 1900	1722 BGAL_EC		m NCBI En	trez:		Description Beta-galactosida	se - Escherichia	a coli (strain K12)			
NCBI BLAST sean Link to retrieve e	ch of 1900	1722 BGAL_EC	COL) equence fro	m NCBI En	trez:			se - Escherichia	ı coli (strain K12)			
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se	ch of <b>POO</b> ntries cont	1722 BGAL_EC	COLI equence from 22/BGAL EC	m NCBI En	trez: Mr(Calc)	Delta(		se - Escherichia RT		Quality	Scan M	. Frag
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides	ch of <b>POO</b> ntries cont	PEAKS( N	IOLI equence from 22 BGAL_EC	m NCBI En	Mr(Calc)	•	Beta-galactosida	RT	Scan			
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpecQNM	ch of <b>POO</b> ntries cont quence	722 BGAL_EC taining this se <u>P0072</u>	COLI equence from 22/BGAL EC	m NCBI En	Mr(Calc) 2 961.47296	Delta( 0.0075	Beta-galactosida	RT				. Frag
NCBI BLAST sear Link to retrieve en Accession/ID Peptides List: ID Se Peptides Peptides Peptides HIT 1YSQ	quence	PEAKS( M 99.12	EOLI equence from 22 BGAL_EC 4/Z 481.74	m NCBI En COLI	Mr(Calc) 2 961.47296 1506.6885	0.0075	Beta-galactosida Error(p File 7.8082 TEST6	RT _2 19.	Scan 523 1056	0.77	73FT-ICR/O.	FT-ICR/0
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides Peptides SpecQNM SpecQNM	quence	PEAKS( M 99.12 99.12	22 BGAL_EC 4/Z 481.74 477.72	m NCBI En COLI Z	Mr(Calc) 2 961.47296 1506.6885 2 953.4277	0.0075	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6	RT _2 19. _2 23.	5can 523 1056	0.77	73 FT-ICR/0. 55 FT-ICR/0.	FT-ICR/0
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpecQNN Ht 1YSQ SpecAPLI	quence I IFNAVR QQL AC[1] DNDI	PEAKS( M 99.12 99.12 99.12	22 BGAL_EC 22 BGAL_EC 4//Z 481.74 477.72 729.36	m NCBI En	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 1456.7158	0.0075 0.0022 0.0104	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6 7.1229 TEST6	RT _2 19. _2 23. _2 23.	Scan 523 1056 085 1189 954 1224	0.77	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O.	FT-ICR/0 FT-ICR/0 FT-ICR/0
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpectPu Hit 1YSQ SpectAPU SpecAPU SpecAPU	quence	PEAKS( № 99.12 99.12 99.12	22 BGAL_EC 22 BGAL_EC 4/Z 481.74 477.72 729.36 633.31	m NCBI En	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 1456.7158 2 1264.6101	0.0075 0.0022 0.0104 0.0046	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6 7.1229 TEST6 3.6681 TEST6	RT _2 19. _2 23. _2 23. _2 27.	Scan 523 1056 523 1056 085 1189 954 1224 142 1356	0.77 0.75 0.78 0.78	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O. 84 FT-ICR/O.	FT-ICR/O FT-ICR/O FT-ICR/O FT-ICR/O
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpecQN SpecQN SpecQN SpecQC	quence que quence que quence que que que que que que que que que qu	PEAKS( M 99.12 99.12 99.12	22 BGAL_EC 22 BGAL_EC 4//Z 481.74 477.72 729.36	m NCBI En	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 1456.7158 2 1264.6101 2 1082.5144	0.0075 0.0022 0.0104	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6 7.1229 TEST6	RT _2 19. _2 23. _2 23. _2 27.	Scan 523 1056 085 1189 954 1224	0.77 0.75 0.78 0.78	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O. 84 FT-ICR/O.	FT-ICR/0 FT-ICR/0 FT-ICR/0
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpectPu Hit 1YSQ SpectAPU SpecAPU SpecAPU	quence que quence que quence que que que que que que que que que qu	PEAKS( № 99.12 99.12 99.12	22 BGAL_EC 22 BGAL_EC 4/Z 481.74 477.72 729.36 633.31	m NCBI En	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 1456.7158 2 1264.6101	0.0075 0.0022 0.0104 0.0046	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6 7.1229 TEST6 3.6681 TEST6	RT _2 19. _2 23. _2 23. _2 27.	Scan 523 1056 523 1056 085 1189 954 1224 142 1356	0.77 0.75 0.78 0.78	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O. 84 FT-ICR/O.	FT-ICR/0 FT-ICR/0 FT-ICR/0 FT-ICR/0
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpectPU Blit 1YSQ SpectPU SpectPU SpectPU SpectPU Hit 6IGLM	quence que quence que quence que que que que que que que que que qu	Z22IBGAL EC     Z22IBGAL EC     Z22IBGAL EC     PO072     PPEAKS( ►     99.12     99.12     99.12     99.12     99.12     99.12	22 BGAL EC 22 BGAL EC 4/Z 481.74 477.72 729.36 633.31 542.26	m NCBI En	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 1456.7158 2 1264.6101 2 1082.5144	0.0075 0.0022 0.0104 0.0046	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6 7.1229 TEST6 3.6681 TEST6	RT _2 19. _2 23. _2 23. _2 27.	Scan 523 1056 523 1056 085 1189 954 1224 142 1356	0.77 0.75 0.78 0.78	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O. 84 FT-ICR/O.	FT-ICR/O FT-ICR/O FT-ICR/O FT-ICR/O
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpectPU Blit 1YSQ SpectPU SpectPU SpectPU SpectPU Hit 6IGLM	quence que quence que quence que que que que que que que que que qu	PEAKS( № 99.12 99.12 99.12	22 BGAL EC 22 BGAL EC 4/Z 481.74 477.72 729.36 633.31 542.26	m NCBI En	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 1456.7158 2 1264.6101 2 1082.5144	0.0075 0.0022 0.0104 0.0046	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6 7.1229 TEST6 3.6681 TEST6	RT _2 19. _2 23. _2 23. _2 27.	Scan 523 1056 523 1056 085 1189 954 1224 142 1356	0.77 0.75 0.78 0.78	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O. 84 FT-ICR/O.	FT-ICR/O FT-ICR/O FT-ICR/O FT-ICR/O
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID See Peptides SpectAPL SpecAPL SpecAPL SpecAPL SpecAPL SpecAPL Hit GIGLN Match	quence I IFNAVR QQL AC[1] QFF QFNISR vC[1] hed peptid	PIOT2           7221BGAL EC           PO072           PEAKS(           P99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.12           99.13	4/2 481.74 477.72 729.36 633.31 542.26	m NCBI En	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 953.4277 2 1264.6101 2 1082.5144 1471.7454	0.0075 0.0022 0.0104 0.0046 0.0089	Beta-galactosida Error(p File 7.8082 TEST6 2.3046 TEST6 7.1229 TEST6 3.6681 TEST6	RT _2 19. _2 23. _2 23. _2 27.	Scan 523 1056 523 1056 085 1189 954 1224 142 1356	0.77 0.75 0.78 0.78	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O. 84 FT-ICR/O.	FT-ICR/O FT-ICR/O FT-ICR/O FT-ICR/O
NCBI BLAST sear Link to retrieve e Accession/ID Peptides List: ID Se Peptides SpecQNN Peptides SpecQNN SpecAPLI SpecAPLI SpecAPLI SpecAPLI SpecAPLI SpecAPLI Match	quence que quence que que que que que que que que que qu	PICLE           7221BGAL EC           taining this se           P0072           99.12	22/BGAL EC 22/BGAL EC 22/BGAL EC 4/Z 481.74 477.72 729.36 633.31 542.26 wenp GV	TOLINRL	Mr(Calc) 2 961.47296 1506.6885 2 953.4277 2 953.4277 2 1264.6101 2 1082.5144 1471.7454 A& HPPFA	0.0075 0.0022 0.0104 0.0046 0.0089	Beta-galactosida           Error(p         File           7.8082 TEST6         2.3046 TEST6           7.1229 TEST6         3.6681 TEST6           8.232 TEST6         8.232 TEST6	RT _2 19. _2 23. _2 23. _2 27.	Scan 523 1056 523 1056 085 1189 954 1224 142 1356	0.77 0.75 0.78 0.78	73 FT-ICR/O. 55 FT-ICR/O. 86 FT-ICR/O. 84 FT-ICR/O.	FT-ICR/O FT-ICR/O FT-ICR/O FT-ICR/O

The "Protein View" collects all the peptide identifications together, summarizes which proteins were present in the sample, and groups homologous proteins together. The same information is displayed in the Peptide View as in this Protein View; however, the results are organized to best enable us to evaluate at the protein level.

This view is helpful when building a summary that can be sent to a customer/collaborator. See chapter 13 for more details on exporting whole files or proteins of interest to an Excel file.

#### Index

The top section of this view (shown above) behaves like an index, listing each protein found in the sample. Very similar proteins, containing the same set or a subset of the matched peptides, are clustered together. To expand and collapse the full list of proteins within each cluster click the '+' or '-' sign respectively.

Accession	The GI, accession or other unique identifier
	for this protein as recorded in the database
	that was searched.
Mass	The calculated mass of this protein
Display	A graphical coverage map. Blue areas
1 0	represent parts of the sequence that have
	been explained by the identified peptides.
Score (%)	A value from 1 to 99 representing the
	confidence we have in this protein
	identification – calculated from the
	confidence on the ten best peptide hits for
	this protein, and normalized against the
	other identified proteins.
Coverage (%)	The number of amino acids in the protein
	sequence that have been explained by the
	identified peptides. Expressed as a
	percentage of the total length of the protein.
Query Matched	The number of spectra explained by
	matching to a peptide from this protein.
Marked	A multi-function checkbox. By default
	unchecked, but we can use this to select
	proteins for export or multiple sequence
	alignment.
Description	The part of the protein's header
-	information as parsed from the database,
	usually it contains the name of the protein.

The table below describes the contents of the columns in the index:

The columns themselves can be customized. Right click anywhere in the report and choose Toggle Column from the pop-up menu. The sub-menu that appears shows a checkmark in each of the columns that are currently showing. Click any of them to show or hide a column. These settings will apply to all your reports.

#### **Sequence Browser**

The "Sequence Browser" tab is selected by default. Clicking on a protein in the index will display the sequence of that protein below in the "Sequence Browser" panel. Clicking on the hyperlink of the accession number of the protein shown in blue will open a new window containing the webpage of the database that you searched for protein ID page in a new window.

NCBI BLAST search of spiQ29443 TRFE_BOVIN Link to retrieve entries containing this sequence from NCBI Entrez:		
Accession/ID	Description	[
sp Q29443 TRFE_BOVIN	Serotransferrin OS=Bos taurus GN=TF PE=2 SV=1	

There is also a "Peptides List" box which displays information about the peptides that matched to the selected protein. This list is identical to the "Peptide View" panel so see this section for more details.

ID	Sequence	PEAKS(	RSD	M/Z	Z	Mr(Calc)	Delta(M	Error( File	RT S.	. Q
Peptides										
<ul> <li>Spectrum 1</li> </ul>	DNPQTHYYAVAVVK	99.0	0.0	802.91	2	1603.7993	-0.0061	3.8056 OrbiSam	0.072	0.78
<ul> <li>Spectrum 2</li> </ul>	TSDANIN[3]WNNLK	99.0	0.0	695.84	2	1389.6523	-0.0132	9.4869 OrbiSam	0.314	0.78
🗉 🧰 Hit 4	HSTVFDNLPNPEDR	99.0				1639.759				
🔹 🌒 Spectrun	5	99.0	0.14	820.89	2	0.0	-0.0065	3.9455 OrbiSam	1.428	0.787
💷 🔶 Spectrun	14	96.64	0.57	547.59	3	0.0	0.0107	6.5511 OrbiSam	1.347	0.777

Below the "Peptides List", you will see the protein sequence with the matching peptide sequences in blue. The darker the blue, the more confident the match is.

Matched peptides shown in blue, SPIDER matches shown in red

- 1 MRPAVRALLA CAVLGLCLAD PERTVRWCTI STHEANKCAS FRENVLRILE
- 51 SGPFVSCVKK TSHMDCIKAI SNNEADAVTL DGGLVYEAGL KPNNLKPVVA
- 101 EFHGTKDNPQ THYYAVAVVK KDTDFKLNEL RGKKSCHTGL GRSAGWNIPM

#### **Sequence Comparison**

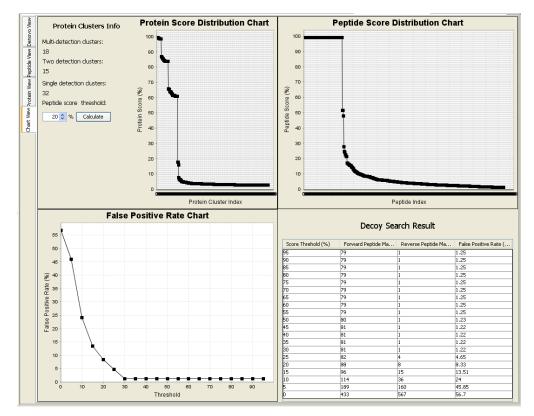
Click on the "Sequence Comparison" tab to open the multiple sequence alignment window. A multiple sequence alignment helps to highlight the differences and similarities between homologous proteins, and the variants you've evidenced from your sample. In the above list of proteins, mark two or more entries by clicking in their checkboxes. Click one of the above buttons to generate the multiple sequence alignment in this frame, or in your web browser.

Identified peptides are highlighted in blue letters on the sequence. A more intense blue indicates a more confident match. The background colors indicate similarity between the sequences. A dark background indicates regions where residues or nucleotides are identical in all sequences, a light background indicates similarity across some sequences, and lowercase letters on white background highlight differences. A dash - is displayed where a gap had to be introduced in one sequence to complete the alignment.

Accession       ID       Mass       Display       PEAX3(Size %)       Coverage(%)       Query matched       Marked       Description         V00001001       V000010000       V000010000       V000010000       V000010000       V000010000       V000010000       V000010000       V000010000       V0000100000       V0000100000       V0000100000       V0000100000       V0000000000       V00001000000000000000000000000000	OrbiSa	mple.mzXML × PEAKS 1 [30-Jan	-09 11:35] ×							
QY441  INF1_JLV6       3       1882/1.52       2.95       0.64       1       Absent in medicinal constraints         Generate Browser       Sequence Browser       Sequence Browser       Sequence Browser       Componention         Comb towser       Doplay infee         Comparison	ew	Accession ID	Mass	Display	PEAKS(Score %)	Coverage(%)	Query matched	Marked		Description
QY441  INFLOG       3       1882/4.52       2.96       0.64       1       Absent in meler         General Browser Doplay nine       Combonser Doplay nine       Combonser Doplay nine       Combonser Doplay nine         QY441  INFE_BOVIN	0 N	DB Search								
QY441  INFLOG       3       1882/4.52       2.96       0.64       1       Absent in meler         General Browser Doplay nine       Combonser Doplay nine       Combonser Doplay nine       Combonser Doplay nine         QY441  INFE_BOVIN	oue l	- 💭 Q29443 TRFE_BOVI	1	77753.2	98.3	33	5.26	4	<b>V</b>	Serotransferrin -
Open browser Display Hime           029443 TRFE BOVIN										
Open browser Display Hilme           029443 TRFE BOVIN           029443 TRFE BOVI	. Viel		3 18	8674.52	2.9	96	0.64	1		Absent in melanc
Open browser Display Hime           029443 TRFE BOVIN	Peptide	Sequence Browser Sequence Co	mparison							
0       029443  TEFE_BOVIN		Open browser Display inline								
0       029443  TEFE_BOVIN	Proteir									
Q29443  TFFE_BOVIN      FAllacavlglc         Q29443  TFFE_BOVIN      FAllacavlglc         Q29443  TFFE_BOVIN		Q29443   TRFE_BOVIN		Pav						
Q29443  TFFE_BOVIN      FAllacavlglc         Q29443  TFFE_BOVIN      FAllacavlglc         Q29443  TFFE_BOVIN	art v	POO330   ADH1_YEAST		siPEt						
P00330 lADH1_YEAST	Ĩ	Q9Y4K1 AIM1_HUMAN	mekrssgrrsgrrrgsqkst	dspgadael <b>PE</b> saarddav	fddevapnaasdnasaekk	vk				
P00330 lADH1_YEAST										
Q9Y4K1  AIM1_HUMAN       spRAaLdggVasaaspeskpspgtkgqlrgesdrskqpppassptkrkgrsraleavpap         Q29443  TRFE_BOVIN      LaDPertvRwctisThEankcAsfREnvLRil		Q29443   TRFE_BOVIN	RAlLacaVlglc							
Q29443  TRFE_BOVIN      LaDPertvRuctisThEankcAsfREnvLRil         Q974K1  AIM1_HUMAN       pasgprapakesppkrVpDEspvtKgtaaeSgEeaarAipRElpVKsssllpeikpehkr         Q29443  TRFE_BOVIN		POO330   ADH1_YEAST								
P00330   ADH1_YHAST		Q9Y4K1 AIM1_HUMAN	sp <mark>RA</mark> aLdggVasaaspeskp:	spgtkgqlrgesdrskqpp	passptkrkgrsraleavp	ap				
P00330   ADH1_YHAST										
Q9Y4K1  AIM1_HUMAN       pasgprapakesppkrVpDPspvtKgtaaeSgEeaarAipRElpVKsssllpeikpehkr         Q29443  TRFE_BOVIN		Q29443   TRFE_BOVIN	LaDPe	ertvRwctisTh <mark>E</mark> ankc <mark>A</mark> s	f <mark>RE</mark> nvLRil					
Q29443  TRFE_BOVIN		POO330   ADH1_YEAST								
P00330   ADH1_YEAST		Q9Y4K1 AIM1_HUMAN	pasgprapakesppkrVpDP	spvtKgtaaeSg <mark>E</mark> eaarAi	.p <mark>RE</mark> lpVKsssllpeikpeh	kr				
P00330   ADH1_YEAST										
Q9Y4K1  AIM1_HUMAN       gplpnhfngraeggrsrelgraagapgasdadglkprnhfgvgrstvttkvtlpakpkhv         Q29443  TRFE_BOVIN		Q29443   TRFE_BOVIN								
Q29443  TRFE_BOVIN		-								
P00330 ADH1_YEAST		Q9Y4K1 AIM1_HUMAN	gplpnhfngraeggrsrelg	aagapgasdadglkprnh	fgvgrstvttkvtlpakpk	hv				
P00330 ADH1_YEAST										
Q9Y4K1 AIM1_HUMAN       elnlktpknldslgnEhnPFsqpVhKgNtatkIslfeNkrtNSsprhtdirgqrntpass         Q29443 TRFE_BOVIN		_	EsgPF	vscVkKtShmdcIkaisNn	eaDAvt					
Q29443  TRFE_BOVIN		-								
P00330 ADH1_YHAST        QkGvifYESQSGvifYES		Q9Y4K1 AIM1_HUMAN	elnlktpknldslgnEhnPF	sqp <mark>VhK</mark> gNtatk <mark>I</mark> slfeNk	rtNSsprhtdirgqrntps	33				
P00330 ADH1_YHAST        QkGvifYESQSGvifYES										
Q9Y4K1 AIM1_HUMAN         ktfvgraklnlakkakemeqpekkvmpnspQnGvLVkEtaietkvtvseeeilpatrgmn           Q29443 TRFE_BOVIN		_								
Q29443   TRFE_BOVIN		_								
F00330   ADH1_YEAST		Q9Y4K1 AIM1_HUMAN	ktfvgraklnlakkakemeq	oekkvmpnspQn <mark>GvLVkE</mark> t	aietkvtvseeeilpatro	mn				
F00330   ADH1_YEAST										
		_			dī	kp				
00VAV1 1 & TM1 HIM&M advance langenged kedvat dega lanvege linvkdhkl lekedeeeede keTv1		_								
Ast With Marken Angender Abdulger School and Astronomic		Q9Y4K1 AIM1_HUMAN	gdssenqalgpqpnqddkad	/qtdagclsepvasalipv	kdhkllekedseaadsks <mark>I</mark>	vl				

#### Chart View

Click on the "Chart View" tab. The following window will appear:



This feature will be described using the data that was chosen for the walkthrough as it is simple data. The "Protein Score Distribution Chart", shows the distribution of the protein scores by percentage. The default peptide score threshold is 20%. In the above example, this threshold results in 32 proteins with a single detection cluster, 15 proteins with two detection clusters and 18 proteins with multi-detection clusters. Modifying the peptide score threshold using the up/down arrows and clicking on the "Calculate" button will result in changes to the amount of clusters that are found for each protein. The "Peptide Score Distribution Chart" displays the scores of the individual peptides as a percentage.

The "False Positive Rate Chart" is derived from running a decoy database search which can be selected from the "Advanced Options" panel when you are setting up your Protein ID parameters.

Advanced Options PEAKS uses a hybrid search technique that requires some sequence tags to help in the I have already run de novo, don't run it again	e search	
Q Run de novo using different parameters than the above	orbisample	~
<ul> <li>Run de novo using the same parameters as above (default)</li> <li>Validation - decoy search</li> </ul>		

The example shown above indicates that below a score threshold of 30%, there is a false positive rate of approximately 1%. More specific details about the false positive rate can be seen in the "Decoy Search Result" table. For example, a score threshold of 20% resulted in 88 matches using a forward database search and 8 matches using a reverse database search. The false positive rate was therefore 8.33%.



# 8. SPIDER Search

After having obtained *de novo* sequences for peptides that are not in the database, it's a good idea to look for a homologous peptide in the database. This will help you to learn more about the proteins in your sample. To search with SPIDER you must first have some good *de novo* sequences.

#### 8.1 Setting up SPIDER Parameters

1) Select a data file or a Protein ID result from the "Project View" frame

2) Click the SPIDER icon on the toolbar  $\Re$  *Or* 

Choose SPIDER Search from the Tools menu.

When a Protein ID	SPIDER Search	X
results file is selected,	Teste	SPIDER Search Save Parameter
the "SPIDER Search	Tools 	
Options" window will appear as seen below:	. Data Refine	Query Options           ③ Segment Match         O Non-gapped Homology Match
uppeur us seen sere w.	. De novo	
	. PEAKS Search	General Options Mass Tolerance (Da):
	√ SPIDER Search	Leucine equals Isoleucine 🔽 Lysine equals Glutamine 🔽
	. PTM Finder	PTM Options
	. Quantification	Name       Mono mass       Residue site         Acetylation (N 42.0106       [ADCEQGILMP]         Acetylation (k)       42.0106       [K]         Amidation       -0.984       [X]@C         Applied Biosys       42.225       [C]         Applied Biosys       227.127       [C]
		Applied Biosys     236.1572     [C]       Show unimod     New PTM     Max variable PTM per peptide:
		Filter Options           Use the spectra which satisfy the following conditions for use in the SPIDER search:           De rovo amino acid score (ALC) greater than:   recommended 0.5
		Protein ID peptide score less than: recommended 0.65 De novo Options
		Advanced Spider search requires sequence tags generated by de novo sequencing for the search           Image: Our sequence of the search           Image: Our sequence of the search
		OK Cancel Help

ools	SPIDER Search Save Parameter orbisample	~
	Query Options	5
Data Refine	Segment Match Non-gapped Homology Match 💿 Homology Match	
De novo		
PEAKS Search	General Options Mass Tolerance (Da): 0.1 Report Top: 1 🗘	
SPIDER Search	Leucine equals Isoleucine V	
PTM Finder	PTM Options	
Quantification	Name         Mono mass         Residue site         Add Fixed =>         Pixed Modification	
	Acetylation (N 42.0106 [ADCEQGILMP A Cetylation (K) 42.0106 [K]	
	Amidation -0.984 [X]@C	
	Applied Biosys 442.225 [C] Add Variable => 🗁 Variable Modification	
	Applied Biosys 450.2752 [C]	
	Applied Biosys         227.127         [C]         <= Remove         Oxidation M           Applied Biosys         236.1572         [C] <td></td>	
	Applied biosys 230.1372 [C]	
	Show unimod New PTM Max variable PTM per peptide: 5 🗢	
	Catabase Options	
	Database options	
	Select Database: swiss vill species	
	New Database Edit Database	
	Set Taxa	
	OK Cancel He	ĺp

If you have selected a data file, the following window will appear:

Note that this window differs from the other window as it asks you to select a does not give you any filter options.

In this case, we assume you already have de novo result for the data file.

By selecting а database, **SPIDER** will search the de novo sequences already generated for that data file that have used the database that you selected.

3) The following section will describe the different options that you have when setting up the parameters for your SPIDER search.

#### Query Options

Choose a Query Type. They are, in order of increasingly rigorous analysis:

Segment Match: this is not a true mutation search, instead, it will insist that the mass of the peptide returned is the same as that of the *de novo* sequence.

*Non-gapped Homology Match:* this search will allow for transpositions, and single point mutations but not insertions or deletions.

*Gapped Homology Match:* this search is the most rigorous, will find all types of mutations, but it is the slowest of the three search modes.

*Block Match:* this is the most rigorous (but most resource intensive) search mode, taking into account all types of mutations and the positional confidence scores. A quick version of this is used to create the reconstructed peptides and to generate the final scores in each of the previous search modes. This is the only search mode that allows you to use variable modifications.

#### General Options

*Amino acid selection:* Choose if you would like PEAKS to consider Leucine equal to Isoleucine without a penalty in the score as well as whether Lysine should be equal to Glutamine without penalty.

*Mass tolerance:* Enter the amount of error (in Daltons) that PEAKS will allow for when determining the peptide sequences.

*Number of peptides to report:* Choose how many of the best homologous peptides should be displayed after searching

#### PTM Options

*PTM Options List:* The "PTM Options" list tells PEAKS what kind of post-translational modifications to include in its analysis. To view additional modifications, select the "Show unimod" box. If your desired PTM does not appear on the list or is different than what is listed, you can select the "New PTM" button and the "PTM Editing" window will open. Fill in the information pertaining to the PTM of interest. For a more in depth explanation of creating a new PTM, see page 53.

To select a PTM as Fixed or Variable, drag the PTM into the "Fixed Modification" or "Variable Modification" box. If you drag over an incorrect PTM, simply drag it back to the "PTM Options" list.

Note that in previous versions of PEAKS, only fixed PTMs were allowed, however PEAKS version 5.0 allows variable PTMs as well when using the new block search.

*Max variable PTMs:* To reduce uncertainty, PEAKS' *de novo* sequencing 'vocabulary' can be limited by restricting the number of variable PTM found on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).

#### Filter Options

As the SPIDER search is computationally intensive, it is not recommended that you run all of your *de novo* sequencing peptides against the database; only those that cannot be well explained.

*De novo score (A.A.) threshold:* The SPIDER search requires a good sequence tag from *de novo* to be able to find good quality homologous proteins. Enter a value for the *de novo* score threshold. The recommended threshold is 0.5.

*Peptide score threshold:* Because there is no need to run SPIDER on peptides that already were found to have a good match during PEAKS protein ID, it is helpful to enter a peptide score threshold so that SPIDER will only be performed on peptides below the threshold. The recommended threshold is 0.65.

#### De novo Options

Because SPIDER requires a *de novo* sequence to find homologous proteins in the database, *de novo* sequencing will need to be performed first. If you have already done *de novo* sequencing, select the "I have already run *de novo*" button.

#### Database Options

Note that these options are only visible if you choose to run a SPIDER search on a data file rather than a PEAKS results file.

*Database to search*: Select from this dropdown list, one of the FASTA databases configured in PEAKS. To edit an already existing database, click on the "Edit Database" button. If the desired database is not in this list, click the "New Database" button. Note that you can also set up a new database in the "Database Configuration" window. The configuration window is the only place that that you can delete databases that you have created. For more information on setting up new databases see page 99.

*Taxonomy selection*: This list displays the taxa you have chosen for your search. If the database selected has taxon information available, you can click on the "Set taxa" button. Otherwise, the whole database will be searched. The selections correspond to established hierarchy -- i.e. selecting 'Mammalia' will search all of 'horse, cow, rat, mouse, human, etc.

4) After setting up parameters, we can save them for future use. Click the "Save Parameters" button, and choose a name for future reference when prompted.

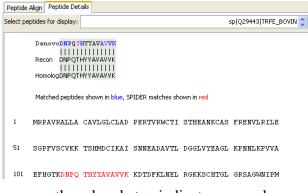
Any parameters that you save will be available in the drop-down list at the top of the window. To see what's inside, just select one, and the parameters boxes will be populated.

5) Press the "OK" button and the SPIDER search will begin.

#### 8.2 SPIDER Results View

SPIDER will search the database for homologous peptides, and attempt to consolidate these into protein hits as well. The result report will look much like the results for PEAKS Protein ID or inChorus searching.

Clicking on the "Peptide View" tab, will display results that look very much like the results for PEAKS Protein ID. See the section on page 55 for more details. Click on the "Peptide details"



tab to see the SPIDER matches shown in red.

Letters on a green background, and with vertical bars, indicate agreement. Letters on a red background indicate sequencing error. Color codes on the *de novo* sequence letters still indicate positional confidence. Letters on a blue background indicate uncertainty or mutation. "+" signs represent more likely mutations. [brackets] indicate an equal mass substitution, common non-critical *de novo* 

errors. <these brackets> indicate an equal mass substitution and a mutation.

When simply identifying exact peptides from the database, using PEAKS Protein ID, or inChorus, there's no need to reconstruct the 'real' sequence.

Clicking on "Protein View" will again yield a similar display as was seen for PEAKS Protein ID (see page 58), however where there were blue regions to indicate areas of homology when performing a protein ID search, there are now red regions to indicate areas of mutation.

SamplE	Data_draft.mzXML × DENOVO 1	[05-Dec-08 14:	38] 🗙 PEAł	<s 1="" 14<="" [05-dec-08="" th=""><th>:39] 🗙 PEAKS 2</th><th>[05-Dec-08 14:44]</th><th>× SPIDER 3 [0</th><th>5-Dec-08 15:06]</th><th>*</th></s>	:39] 🗙 PEAKS 2	[05-Dec-08 14:44]	× SPIDER 3 [0	5-Dec-08 15:06]	*
View	Accession	ID Mas	s	Display	SPIDER(Score	Coverage(%)	Query matched	Marked	Description
	DB Search								
Denovo	🚊 🔘 Q29443 TRFE_BOVIN	1	77753.2		1	5.26	4		Serotransferrin
	Q4L5U1   RIMM_STAHJ	21	19309.496		1	7.78	1		Probable 165 rR
View	BOTW44 GIDB_FRAP2	9	23253.205		1	6.37	1		Methyltransfera
le <	Q8AA41 ISPE_BACTN	11	30533.79		1	4.74	1		4-diphosphocyti
beptide	- 💭 P19231   Y32K_BNYVG	28	31869.223		1	4.26	1		RNA-4 uncharac
	Q15N42 NHAA2_PSEA6	17	42666.598		1	4.24	1		Na(+)/H(+) anti
View	Q8D666 NHAA2_VIBVU	18	48896.16		1	4.03	1		Na(+)/H(+) anti
	Q5X484 METN_LEGPA	13	37639.16		1	3.81	1		Methionine impo
Protein	O P25033 HEMO_HYACE	10	45648.945		1	3.15	1		Hemolin precurs
لق	⊕ O A3PTR0 NHAA1_MYCSJ	14	66444.64		1	3.1	3		Na(+)/H(+) anti
	P49046 LEGU_CANEN	12	52762.76		1	2.95	1		Legumain precu
	Q95220 RECN_STRCO	19	59837.258		1	2.62	1		DNA repair prot
		22	63625.293		1	2.45	1		Mitochondrial im

Click on the "Sequence Browser" tab, and note that instead of highlighting areas of homology in blue, areas of mutation are highlighted in red.

Sequenc	e Browser Sequence	ce Comparison				
ID	Sequence	SPIDER M/Z	Z	Mr(Calc) I	Delta(M Er	rror(p File
Pep 📄	otides SpectGSTVFDN	16.0	820.89	2 1518.706	-121.0594	73827.3 Samp
						·
	Matched peptide	es shown in <mark>blue, re</mark>	ed for SPIDER:			
1	MQVEVGQIVN	THGIKGEVKV	KSNSDFTDTR	FQPGEVLTVN	HONHEEOI	JTV
51	LSYRVHKGFH	MLKFEGINNI	NDVEQYKGDY	LYQERDHEDI	ELAENEY	YYS
101	DIIGSTVFDN	DNQPIGRVIN	IFETGANDVW	VVKGEKEYLI	PYIADVVI	KEI
151	DIENKTIRIT	PMEGLLD				

After finding a homologous peptide in the database, SPIDER will decide what is likely a mutation and what is more likely a simple *de novo* sequencing error (resulting from certain combinations of amino acids having exactly the same mass – L/I, N/GG, AG/G, etc.). As such it reconstructs the 'real' sequence from a *de novo* sequence and its homologue. This is highlighted on the "Peptide Details" frame of "Peptide View".

# Chapter 9

# 9. PTM Finder

# 9.1 Setting up PTM Finder Parameters

1) Select a Protein ID results file to perform a PTM finder search on. Note that you cannot perform protein ID on a raw file or *de novo* results.

2) Click the PTM icon on the toolbar  $\overset{\triangleleft}{\sim}$ 

Ór

Select "PTM Finder" from the Tools menu.

The "PTM Finder Options" window will appear:

	PTM Finder		Save Parameter	orbisample	ľ
	Mass Options			General Options	
Refine	Parent Mass Error Tolerance:	0.1 Da 💙 Pi	recursor Mass Search Type:	Preprocess this data 'on I	the flut
			recarsor mass bearch rype.		
vo	Fragment Mass Error Toleran	:e: 0.8 Da 🔽 🤇	Monoisotopic O Average	Max Missed Cleavages:	1 🌲
5 Search	Enzyme Options				
5 Sodren	Enzyme:	Trypsin		New En	zvme
R Search	,				-,
are bodi en	Digest Rule:	RK	{P}	Delete E	nzyme
inder	Find peptides that satisfy	the above rule at both end	ds	Adva	nce
tification	PTM Options				
	Name Mono ma	ss Residue site	Add Fixed =>	Fixed Modification	
	4-hydroxynon 156.115	[CHK]			
	Homoserine -29.9928	[M]@C	<= Remove		
	Homoserine lac48.0034	[M]@C			
	Hydroxylation 15.9949	[PKDNRY]			
	ICPL-heavy 111.0416	[K], [X]@N			
	ICPL-light 105.0215	[K], [X]@N			
	Iodoacetic acid 58.0055	[C]			
	Lipoyl 188.033	[K]			
	Methyl ester 14.0156	[DE], [X]@C			
	Methylation 14.0156	[CKRHDENQ],			
	O18 label 2.0042	[STY], [X]@C	Add Variable =>	Variable Modification	
	Propionamide 71.0371	[C]			
	Trimethylation 42.047	[CKRHDENQ],	<= Remove		
	Myristoylation 210.1984	[KC], [G]@N			
	N-acyl diglyceri 788.7258	[C]	-		
	N-isopropylcar 99.0684	[C]			
	N-Succinimidyl 127.0633	[K], [X]@N			
	Oxidation M 15.9949	[K], [K]@N [M]			
	Oxidation HW 15.9949				
		[HW]			
	Palmitoylation 238.2297	[CSTK]	<b>⋎</b>		
	Show unimod	New PTM		Max variable PTM per peptide:	3 🛟
	Filter Options				
	Filter the spectra which sat	sfy the following condition	is for use in the PTM search:		
	De novo amino acid score g	reater than: 0.5 rec	ommended 0.5		
	Protein ID peptide score les	s than: 0.65 reco	ommended 0.65		

The parameters are the same as you used when performing protein ID (page 51) with the exception of the filter options found at the bottom of the window. As PTM Finder searches tend to be computationally intensive, PEAKS will only look at *de novo* sequencing results that are above the amino acid score threshold and below the peptide score threshold that you input.

*De novo score (A.A.) threshold:* The PTM finder requires a good sequence tag from *de novo* to be able to find good quality homologous proteins. Enter a value for the *de novo* score threshold. The recommended threshold is 0.5.

*Peptide score threshold:* Because there is no need to run the PTM finder on peptides that were already found to have a good match during PEAKS protein ID, it is helpful to enter a peptide score threshold so that SPIDER will only be performed on peptides below the threshold. The recommended threshold is 0.65.

#### 9.2 PTM Finder Results View

The results from a PTM finder search are identical to those seen in a PEAKS Protein ID search. Please see page 55 for more information on the PEAKS Protein ID search results.



# **10. inChorus Meta Search**

inChorus Protein Identification will call upon several search engines for protein identification and will then compare and summarize the results from the different search engines in one single report. PEAKS protein ID, X!Tandem, OMSSA, Mascot and Sequest. Please note that you will need to have your own copy of Mascot and Sequest in order to make use of those search engines during and inChorus search. In order to set up your search engine preferences, see page 93.

#### **10.1 Setting up inChorus Parameters**

1) Select the orbisample.mzxml file

2) Click the "inChorus Search" icon on the toolbar O *Or* 

Select "inChorus Search" from the Tools menu.

The "inChorus Options" window will appear:

InChorus Search		
Tools		
PEAKS Protein ID		
X!Tandem		
OM55A		
Mascot		
Sequest		
🔲 Import Result		
	OK Cancel	Help

3) First select each of the protein identification tools that you would like to use by putting a checkmark in their respective checkboxes. Search parameters for each program can be set by selecting the name of the search engine. You will need to use the "Ctrl" button to be able to check the boxes for multiple search engines.

The option screens for each of the search engines available to inChorus are designed to work in the same way as options screens for the original programs. For help in setting search parameters for each program, please refer to that program's user manual. For help with PEAKS Protein ID, please refer page 51.

#### Importing Existing Results

PEAKS inChorus reads X!Tandem .xml files, OMSSA .omx files, Mascot .dat files and Sequest .srf files. When importing third party results files, please make sure that the scan number model in the results file is consistent with the one in PEAKS. PEAKS uses original data information to compute the inChorus score. When you run X!Tandem search with command line, you need to turn on the option of ``-w`` in order to export data information into X!Tandem .xml files.

To import existing results, check the "Import Result" checkbox and select "Import Result". The following window will open.

InChorus Search	X
Tools	Import Result
PEAKS Protein ID XITandem OMSSA Mascot	Database         Define a common database for importing search result.         Database to search         Swiss         Taxonomy selections         all species         Set Taxa
Sequest	Import Search Result
Import Result	Import XTandem .xml file Browse
	Import Omssa .omx file Browse
	Import Mascot .dat file Browse
	Import Sequest .srf file Browse
	OK Cancel Help

Although it is not necessary for the various search engines to use the same database in an inChorus search, it is necessary to have a unified database for an inChorus search that includes imported results. Select the database that you would like to use from the dropdown list. The inChorus search will be performed on all species in the database unless specified by the user. If this database does not appear in this list, refer to page 99 to configure your databases. To specify which taxa you would like to search, click on the "Set Taxa" button. You will need to use the "Ctrl" key to make multiple selections.

To import your file, click the "Browse" button that is found beside the appropriate search engine. Find the file that you would like to import and click "Open". Once you have selected the file(s) that you would like to import and have selected the options for any other search engine searches you would like to perform, click "OK".

## **10.2 inChorus Results View**

When the inChorus search is complete the "Project View" panel should contain a separate results file for each search engine that you selected as well as an inChorus report that combines the results from the multiple search engines. See an example below:

PEAKS 3 [12-Jan-09 14:25]
 XI XTANDEM 4 [12-Jan-09 14:26]
 OMSSA 5 [12-Jan-09 14:28]
 INCHORUS 6 [12-Jan-09 14:28]

#### De novo, Peptide and Protein Views

Each results file for the  $3^{rd}$  party search engines looks very similar to the PEAKS protein ID results file (page 55) with a few small differences. Firstly, there is no "*De novo* View" or "Chart View", and secondly, the scoring will be specific to that search engine. For example, the score for OMSSA is listed as an E-value. For more information about different scoring methods, refer to the user manual of the  $3^{rd}$  party search engines.

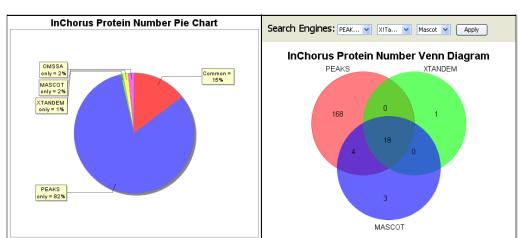
The inChorus search report also looks very similar to the 3<sup>rd</sup> party search engine results, however there are no "*De novo* View" and "Chart View" results available. The *de novo* sequencing results that are found in the "*De novo* View" are only those that correspond to results that have been identified by one of the search engines in the inChorus search.

#### Chart View

As mentioned above, "Chart View" is available in the inChorus report. The two charts that appear at the top, the "Protein Score Distribution Chart" and the "Peptide Score Distribution Chart" are in the same format to those that are seen in the "Chart View" of a PEAKS Protein ID search (page 61).

In the example below, the "inChorus Protein Number Pie Chart" displays the percentage of identified proteins that were found by PEAKS, OMSSA, Mascot and X!Tandem. PEAKS identified 82% of the proteins on its own and 15% of proteins were common between some of the search engines. The "inChorus Protein Number Venn Diagram" gives more specific information than the pie chart about the overlap of the results between different search engines. The Venn diagram contains information about 3 search engines and inChorus was run using 4 search engines for this example. You can change the search engines that will appear in the Venn

diagram using the dropdown lists. Select the 3 search engines that you would like to compare and click the "Apply" button.



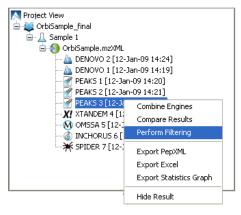


# **11. Filtering Your Results**

PEAKS 5 provides you with an exhaustive list of all proteins and peptides that can be found in a sample. However, since everyone has their own criteria of what information is required in their report and what is an acceptable result, PEAKS 5 provides the necessary filtering tools that enable you to filter out the less critical information and leave you with the essentials.

#### **11.1 Setting Filter Parameters**

Click on the time and date stamp associated with the result that you would like the filter. Once the report loads, click the right button on your mouse and select "Perform Filtering".

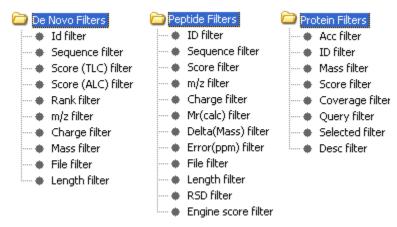


The following window should appear:

🔼 Data Refine		
Tools ✓. Result Filtering	De Novo Filters Peptide Filters Protein Filters	De Novo Filters     Poptide Filters     Protein Filters
	Filter Options         De novo view shows peptides that could not be explained by proteins from the Peptide View         De novo view shows all peptides that are not filtered         Remove de novo peptides with no matching         database results         Parameter Options         Set saved as default       Clear default         Default Parameters:	Edt Filter
	OK Cancel	Help

#### Possible Filters/ Selected Filters/ Edit Filter

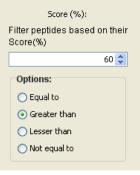
The filters are grouped into three basic types to reflect what they act on: *De novo* filters act to remove proposed *de novo* sequences; Peptide Filters act to remove peptides found in the database from the report; and Protein filters act to remove proteins from the report. To see the available filters for each level of filtering, double click on the appropriate folder in the "Possible Filters" frame. See the options in each folder below:



Choose a filter from the 'Possible Filters' list on the left by clicking on it. Options for this filter will appear in the "Edit Filter" frame. Once you have set the options that you would like for the filter in the "Edit Filter" frame, drag the filter that you would like into the "Selected Filter" list on the right hand side. Click "OK" to apply the filter that you have selected to the current file.

If you would like to add another filter, you can repeat the process, continuing to add as many filters as necessary. In this way it is also possible to have two filters on the same property; we can set a range of protein mass, for instance, by applying one filter on the upper bound of the mass and adding another filter to be the lower bound of the mass. We can also have more complex filters that involve multiple properties.

For example, let's say that you want to show only proteins with more than one high scoring (greater than 60% score) peptide, a standard requirement for publication. Double click on the "Peptide Filters" folder. Select "Score Filter". Edit the filter to select peptides that have a score that is greater than 60% in the "Edit filter" frame.



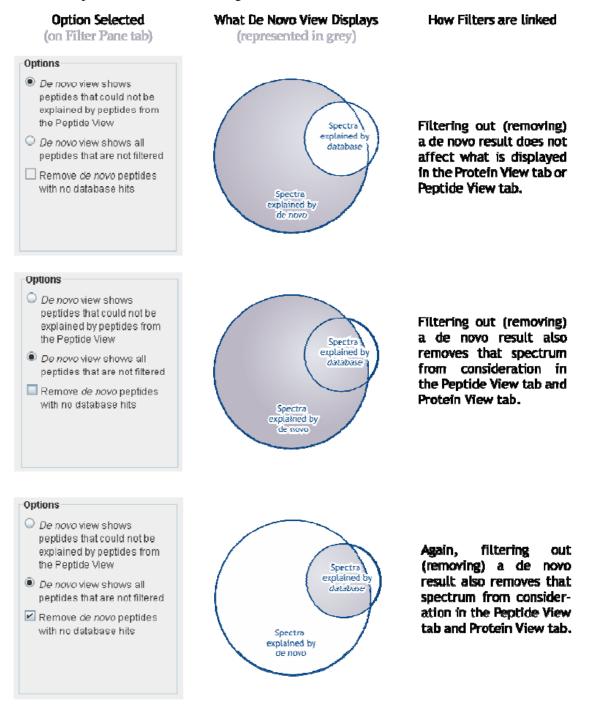
Selected Filters De Novo Filters

Peptide Filters
 Score filter: > 60.0
 Protein Filters

Now drag "Score filter" from the "Possible Filters" frame to the "Selected Filters" frame.

#### Filter Options

The filters cascade through each view in the multi-part report. For instance, removing a peptide from the database search results – the Peptide View list – will cause the *de novo* sequence for that peptide to be removed from the "*De novo* View" tab but will not affect the "Protein View" list. Filtering out a protein (by mass, for example) will remove it from the "Protein View" list and will remove all peptides associated with that protein from the "Peptide View" list as well as from the "*De novo* View" list. The manner in which the "*De novo* View" is linked can be specified by the user using the options in the "Filter Options" frame. See the figure below for more information.



#### Parameter Options

Filter sets can be saved and re-used between sessions, by clicking the "Save Parameter" button that is found at the top right hand corner of the "Filter Parameters" window.

You may prepare your results the same way each time; in which case it makes sense to set up a filter that will be automatically applied each time we load a report. Select a filter from the list of saved filters from the dropdown menu found at the top right hand corner of the "Filter Parameters" window. Click on the "Set saved as default" button. This filter will be displayed in the "Parameter Options" frame (as seen below) and will be applied automatically just after a report is loaded. Be careful, if your default filter is very stringent, it can sometimes

remove everything! To remove a default filter, press the Clear Default Button at the bottom of the Filter Pane.

Parameter Options
Set saved as default
Clear default
Default Parameters: orbisample

Each filter can be applied several times over. So it can get a little complex. To illustrate, here are a few examples:

1) Goal: Show proteins that have two high-scoring hits:

-Add the Protein Filter called "Query" and in the "Edit Filter" section choose 'greater than' and type '1' in the box (without the quotes). This will remove any 'one hit wonders'.

-Add the Peptide Filter called 'Score' and in the "Edit Filter" section choose 'greater than' and type '50' in the box (without the quotes).

2) Goal: Find a protein that contains the word 'human' or 'rat' in the database entry's description, but not Keratin or Trypsin.

-Add the Protein Filters called 'Desc'

-In the "Edit filter" section, you are required to type in a regular expression (regex). This allows you to use wildcards.

Wildcard	Meaning	Example
.*	"Anything of any length"	<b>.*human.*</b> Will find anything that contains the word 'human', with anything before and anything after.
Ι	"Or" (use brackets)	.*(human rat RAT).* Will find anything that contains the word 'human', or the word 'rat' or the word 'RAT', with anything before and anything after.
?!	"Not" (use brackets)	(?!.*(Keratin Trypsin).*).*(human rat).* will find anything containing human or rat but not Keratin or Trypsin
[]	"Any of these characters"	.*([Hh]uman [Rr]at).* will find anything containing the words Human, human, Rat or rat.

-So type in the regex: (**?!.\*(Keratin|Trypsin).\*).\*(human|rat).\*** and press the Enter key. If PEAKS confirms that this is a valid regular expression, it will put a check in the 'Valid Java Regex' box .

3) Goal: Setting a protein mass range

If we know the approximate mass of the proteins you are interested in, you can eliminate all proteins that are not close in mass.

Add two filters: "Protein Filters: Mass >12000" and "Protein Filters: Mass < 32000".



# 12. Complex Analysis

## 12.1 Creating a project for complex system

PEAKS 5 is able to analysis MS data from very complex systems. The data analysis scheme is organized as follows:

Project nodes

 $\square$  Sample nodes

File nodes

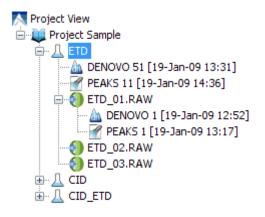
Below is an example of a project that contains three samples that were generated using the following fragmentation methods: ETD, CID, and CID/ETD. Each sample has three files.

🔼 PEAKS	
File Tools Window Help	
	. 💩 📝 🗡 💐
Project View thermo data CID CID_01.RAW CID_02.RAW CID_02.RAW CID_03.RAW ETD ETD_01.RAW CID_03.RAW CID/ETD_03.RAW CID/ETD_01.RAW CID/ETD_03.RAW CID_ETD_03.RAW	
Tasks Running Info Properties	
Selection Details: thermo data	
Project Name Total Samples	thermo data 3

#### 12.2 Integrating data analysis

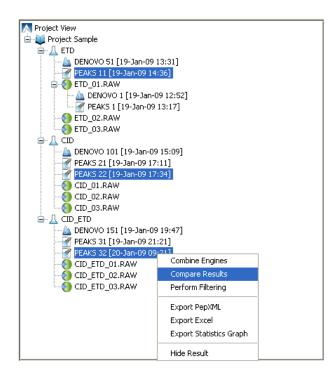
Within a project, the data is analyzed either file by file or sample by sample. By selecting a sample, the operation applies to all files in the sample. It means all spectra from different files are processed in a single run. The result node is at same level as selected data node.

PEAKS 1 is the PEAKS Protein ID result for file ETD\_01.RAW, whereas PEAKS 11 is the PEAKS Protein ID result for all three fractions of the ETD sample. Note that the result of a sample may not be the sum of the results of all files in the sample.



# 12.3 Comparing results

PEAKS 5 provides a "Compare Results" function to align/differentiate two or more results. To use the "Compare Results" function, hold down the "Ctrl" key and select two or more result files that you would like to compare. Click on the right mouse button and select "Compare Results".



Below you will see a comparison of the PEAKS protein ID results (PEAKS 11, PEAKS 22 and PEAKS 32) generated for the three samples mentioned in the previous section: ETD, CID and CID\_ETD.

After selecting the "Compare Results" function for PEAKS 11, PEAKS 22 and PEAKS 32, a new entry will appear in the "Project View Frame" called Compare PEAKS 11, 22, 32.

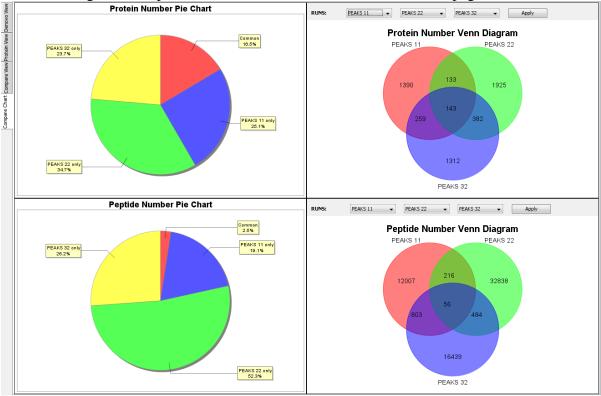
Note that the results window that appears will contain "*De novo* View", "Protein View", "Compare View" and "Compare Chart" tabs. In the "Protein View", all proteins found by each of the in the result of PEAKS 11 PEAKS 22 or PEAKS 32 are listed in the first column. For each protein, the scores in different database search results are displayed.

5 32 [20-Jan-09 09:21] 🗙 Compare	11,22,32 ×			
Accession	PEAKS 11 P	EAKS 22	PEAKS 32 Ma	ss Description
DB Search				
Q93615 ETFA_CAEEL			99.14	34454.562 Probable electron transfer flavoprotein subunit alpha, mitochondrial OS=Caenorhabditis elegans GN=F27D4.1 PE=2 SV
Q91717 CO2A1_XENLA		88.25		142263.17 Collagen alpha-1(II) chain OS=Xenopus laevis GN=col2a1 PE=2 SV=2
Q17967 PDI1_CAEEL			99.15	53436.16 Protein disulfide-isomerase 1 OS=Caenorhabditis elegans GN=pdi-1 PE=2 SV=1
			99.14	38659.105 605 ribosomal protein L4 OS=Caenorhabditis elegans GN=rpl-4 PE=1 SV=3
— Q6P4Z2 CO2A1_XENTR		82.97		142695.94 Collagen alpha-1(II) chain OS=Xenopus tropicalis GN=col2a1 PE=2 SV=1
P02566 MYO4_CAEEL	99.14	64.92	99.15	225124.52 Myosin-4 OS=Caenorhabditis elegans GN=unc-54 PE=1 SV=1
Q9XXK1 ATPA_CAEEL	99.14	17.47	99.15	57787.613 ATP synthase subunit alpha, mitochondrial OS=Caenorhabditis elegans GN=H28O16.1 PE=1 SV=1
P46561 ATPB_CAEEL	99.14	26.98	99.15	57526.82 ATP synthase subunit beta, mitochondrial OS=Caenorhabditis elegans GN=atp-2 PE=1 SV=2
P10567 MYSP_CAEEL	99.14	61.09	99.15	101949.57 Paramyosin OS=Caenorhabditis elegans GN=unc-15 PE=1 SV=1
			99.12	24312.72605 ribosomal protein L6 OS=Caenorhabditis elegans GN=rpl-6 PE=2 SV=1
			87.52	8712.084 ATP synthase subunit beta (Fragment) OS=Streptococcus downei GN=atpD PE=3 SV=1
-O P46563 ALF2_CAEEL			99.15	38846.258 Fructose-bisphosphate aldolase 2 OS=Caenorhabditis elegans GN=F01F1.12 PE=1 SV=1
			99.12	60101.055 Chaperonin homolog Hsp-60, mitochondrial OS=Caenorhabditis elegans GN=hsp-60 PE=2 SV=2
A9MR77 DNAK_SALAR		14.03	79.54	69319.234 Chaperone protein dnaK OS=Salmonella arizonae (strain ATCC BAA-731 / CDC346-86 / R5K2980) GN=dnaK PE=2 SV=1
P20442 DNAK CAUCR		59.09	82.7	67615.73 Chaperone protein dnaK OS=Caulobacter crescentus GN=dnaK PE=2 SV=2

Similarly, in "Compare View", all peptides in the result of PEAKS 1 or PEAKS 5 are listed in the first column. For each peptide, the spectrum id, m/z and score in different database search results are displayed.

Sequence	PEAKS 11 Spectrum Id	PEAKS 11 MZ	PEAKS 11 Score	PEAKS 22 Spectrum Id	PEAKS 22 MZ	PEAKS 22 Score	PEAKS 32 Spectrum Id	PEAKS 32 MZ	PEAKS 32 Score
LDATVHGEVSSK	Spectrum 6875	621.82	16.68		1		Spectrum 38495	621.82	87.5
ATGVLYDYVNK	Spectrum 6875	621.82	4.63						
NNDKKN[2]N[2]K	Spectrum 2	488.73	5.48						
EVKN[2]N[2]ENK	Spectrum 2	488.73	4.84						
IGGIGTVPVGR	Spectrum 3890	513.31	42.88	Spectrum 10175	342.54	13.64	Spectrum 29859	513.31	98.9
VEAPPAKVSK	Spectrum 6964	513.31	4.08	Spectrum 10175	342.54	8.49			
SPQ[2]SGTN[2]KK	Spectrum 4	474.74	5.56						
EKSN[2]N[2]NIK	Spectrum 4	474.74	2.81						
QEYDESGPSIVHR	Spectrum 5	506.24	88.14				Spectrum 29620	506.24	67.94
IIKEN[2]LGRSAM[3]YR	Spectrum 6	784.41	2.71						
DFN[2]VEYIQRGGLR	Spectrum 5282	784.39	12.1						

The Compare Chart provides Venn diagrams and pie charts for proteins and peptides to illustrate the comparison of results that were generated by each protein ID search. For more information on the Venn diagrams and pie charts in PEAKS's "Chart View" refer to page 61.



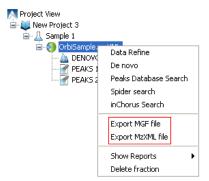


# 13. Exporting Data/Reports and Printing

PEAKS 5 allows you to create reports to share with collaborators, colleagues and clients. The reports are available in HTML or Microsoft Excel (.xls) formats and follow a 'What you see is what you get' philosophy. All the information you see on screen in PEAKS 5 will appear in the exported report. For this reason, it is important that we complete results filtering and toggling columns before exporting a report.

# 13.1 Export Data in .mzxml or .mgf

In order to export your data file in .mzxml or .mgf right click on the data file that you wish to export.



Click "Export MGF File" A window will open that will prompt you to enter a name and a location for the file. Click "Export".

or

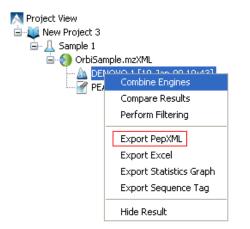
Click "Export MzXML File". The following window will open:

🔼 Exprot MzXML File	×
Start RT:	End RT:
Save as:	Browse
ОК	Cancel

Enter the start and end RT in the appropriate boxes. Then click the "Browse" button to select a destination to save your file.

# 13.2 Export Peptide Results in PepXML Format

In order to export your PEAKS results file in PepXML right click on the results file that you wish to export and select "Export PepXML".



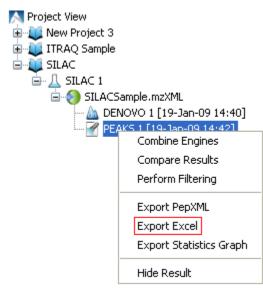
The following window will appear:

🔼 Export to	PepXML Specification	X
←Select the Pep> PepXML File:	(ML Export Destination	Browse
	OK Cancel	Help

Browse your computer to select the location that you would like to export the PepXML file to. Then click "OK".

# 13.3 Export Results in Excel Format

In order to export your PEAKS results file to Excel right click on the results file that you wish to export and select "Export Excel".



The following window will appear:

Export Excel Result Report		
Select the Type of Results to Export		
<ul> <li>Currently Highlighted Protein and Corresponding Peptide(s)</li> </ul>		
🔿 Complete Protein List (Peptide Details Omitted)		
Marked Protein(s) and Corresponding Peptide(s)		
All Protein and Peptide Result(s) (one representative protein per group)		
<ul> <li>All Protein and Peptide Result(s)</li> </ul>		
Options:		
Export Data Properties		
Export Search Parameters		
Export Filter Conditions		
Select the Export Destination		
Excel File: Browse		
OK Cancel Help		

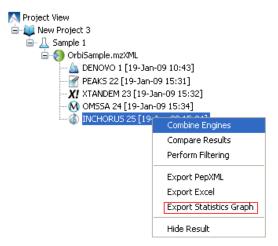
If you would like to export all of the protein and peptide results select the "All Protein and Peptide Result(s)", otherwise select one of the other options where you can limit which results are exported. Select the appropriate boxes if you would like to export "Data Properties", "Search Parameters" and "Filter Conditions" to Excel. Finally you need to select the "Export Destination" by clicking the "Browse" button. Then click "OK".

If you export *De novo* results to Excel, the *de novo* sequencing results will be exported and you have the choice to also export "Data Properties", "Search Parameters" and "Filter Conditions". See below:

Export Excel Result Report	×
Select the Type of Results to Export	
Export Data Properties	
Export Search Parameters	
Export Filter Conditions	
L	
Select the Export Destination	
Excel File:	Browse
	OK Cancel Help

#### 13.4 Print Tables and Graphs for Publication

In order to export an image file, right click on the results file that contains the appropriate image file and select "Export Statistics Graph".

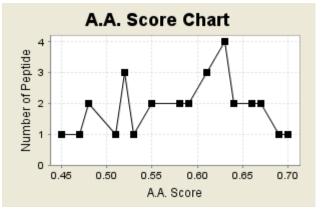


#### De novo Image Files

Select a *De novo* results file. Right click and select "Export Statistics Graph". The following window will appear:

🔼 Export Statistics Graph Specification	
Statistics Graph Type	
💿 De novo A. A. Score Chart	O Protein Score Chart
O Peptide Score Chart	○ False Positive Rate Chart
🔿 InChorus Result Pie Chart	🔿 InChorus Result Venn Diagram
O Peptide Number Pie Chart	🔿 Peptide Number Venn Diagram
O Protein Number Pie Chart	🔿 Protein Number Venn Diagram
Options:	
File Format: jpg 💙 Width:	300 🗘 Height: 200 🗘
Select Export File Destination	
Terrer Eiler	
Image File:	Browse
	OK Cancel Help

The "*De novo* A. A. Score Chart" option will be selected in the "Statistics Graph Type" panel. Select a file format and height/width for your chart and browse your computer to select a destination. Beside is an example of the output:

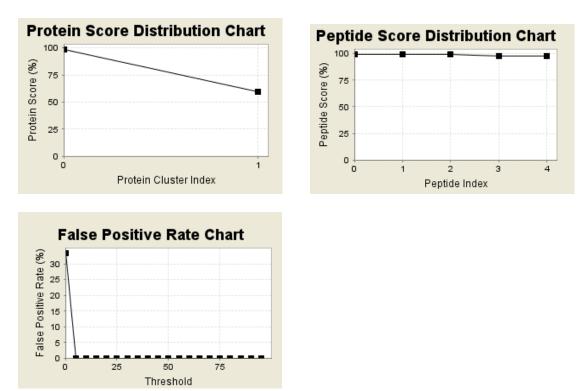


#### **Protein ID Image Files**

Select a Protein ID results file. Right click and select "Export Statistics Graph". The following window will appear, giving you the option of exporting a "Protein Score Chart", a "Peptide Score Chart" or a "False Positive Rate Chart:

Protein Score Chart
◯ False Positive Rate Chart
🔿 InChorus Result Venn Diagram
🔿 Peptide Number Venn Diagram
O Protein Number Venn Diagram
300 🗘 Height: 200 🗘
Browse
OK Cancel Help

Below are examples of the "Protein Score Chart", the "Peptide Score Chart" and the "False Positive Rate Chart", respectively.

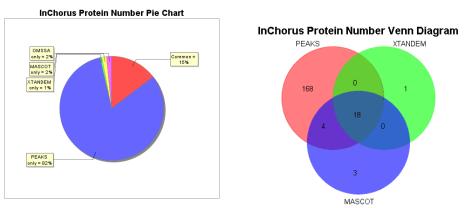


#### inChorus Image Files

Select an inChorus results file. Right click and select "Export Statistics Graph". The following window will appear, giving you the option of exporting a "Protein Score Chart", a "Peptide Score Chart", an "inChorus Result Pie Chart" or an "inChorus Result Venn Diagram".

🔼 Export Statistics Graph Specification	
Statistics Graph Type	
🔵 De novo A. A. Score Chart	Protein Score Chart
O Peptide Score Chart	◯ False Positive Rate Chart
🔿 InChorus Result Pie Chart	🔿 InChorus Result Venn Diagram
O Peptide Number Pie Chart	🔿 Peptide Number Venn Diagram
O Protein Number Pie Chart	O Protein Number Venn Diagram
Options:	
File Format: png 💽 Width:	300 🔷 Height: 200 🔷
Select Export File Destination	
Image File:	Browse
	OK Cancel Help

Below are examples of the "inChorus Result Pie Chart" and "inChorus Result Venn Diagram", respectively.



Examples for the "Protein Score Chart" and the "Peptide Score Chart" can be found above in the "Protein ID image files" section.

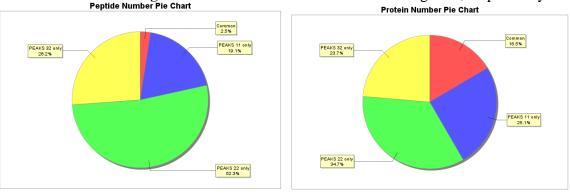
#### **Compare Image Files**

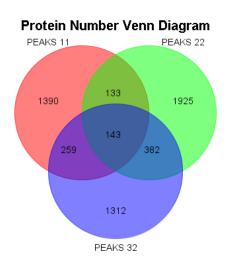
Select a "Compare" results file (see chapter 12 for more information on comparing results files). Right click and select "Export Statistics Graph". The following window will appear, giving you

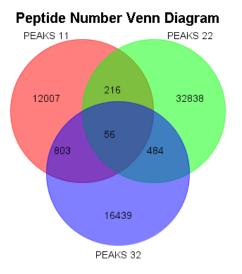
the option of exporting a "Peptide Number Pie Chart", a "Protein Number Pie Chart", a "Peptide Number Venn Diagram" or a "Protein Number Venn Diagram".

🔼 Export Statistics Graph Specification	X
Statistics Graph Type	· · · · · · · · · · · · · · · · · · ·
🔵 De novo A. A. Score Chart	O Protein Score Chart
O Peptide Score Chart	◯ False Positive Rate Chart
🔿 InChorus Result Pie Chart	🔿 InChorus Result Venn Diagram
Peptide Number Pie Chart	O Peptide Number Venn Diagram
🔿 Protein Number Pie Chart	🔿 Protein Number Venn Diagram
Options:	
File Format: png 🛛 💙 Width:	300 🗘 Height: 200 🗘
Select Export File Destination	
Image File:	Browse
	OK Cancel Help

Below are examples of the "Peptide Number Pie Chart", the "Protein Number Pie Chart", the "Peptide Number Venn Diagram" and the "Protein Number Venn Diagram", respectively. Peptide Number Pie Chart









# 14. Advanced Configuration and Environment Preferences14.1 PEAKS Environment Preferences

This section will describe the setting up the configuration of environmental preferences including general, instrument, search engine and ion editor configurations.

To begin click the Preferences toolbar icon  $\bigcirc$ Or

Select "Preferences" from the "Window" menu.

The following window will open:

📐 Preferences		
∃ General	General	
Instrument	Defends Jacob File Directory	
Search Engine	Default Input File Directory	
🗉 Ion Editor	\\192.168.1.30\data\LTQ-FT - RAW - Juan Casado	
		Browse
	Default Output Directory	
	Output Directory	Browse
	C:\derbyServer	
	Project folder	Browse
	C:\derbyServer\serverDB	
	Temporary File Directory	
	C:\temp_mgf	
		Browse
	Default Configuration File Directory	
	C:\Documents and Settings\bsi\.peaks\peaksconf.xml	
		Browse
	Default Log File Location	
	C:\Documents and Settings\bsi\.peaks\peaks.log	
		Browse
		Apply

Use the '+' and '-' boxes to expand and collapse the view.

#### **General Preferences**

*Default Input File Directory-* Select where your data is being inputted from using the "Browse" button.

*Default Output File Directory*- PEAKS outputs your results to C:\derbyServer by default. Select the "Browse" button to change this location.

*Project folder*- PEAKS uses C:\derbyServer\serverDB as the default project folder. Select the "Browse" button to change this location.

*Temporary File Directory*- PEAKS uses C:\temp\_mgf as the default project folder. Select the "Browse" button to change this location.

*Default Configuration File Directory*- Your configuration files for PEAKS can be found at C:\Documents and Settings\bsi\.peaks\peaksconf.xml by default. Select the "Browse" button to change this location.

*Default Log File Location*- Your log file for PEAKS can be found at C:\Documents and Settings\bsi\.peaks\peaks.log by default. Select the "Browse" button to change this location.

#### **RMI** Connections

Clicking on "RMI connections" on the menu on the left hand will open the following window:

A Preferences			
🖃 General	RMI Connecti	ons	
	Server Host	localhost	
Instrument	Server Port	3003	
⊞ Search Engine	Client Port	1000	
⊞ Ion Editor	Worker Port	5000	

The default port numbers for the Server, Client and Worker will appear. The port numbers can be changed if conflicts arise. Contact technical support at BSI for more information.

Click the "Apply" button to save any changes that you made.

#### **Derby Database**

Clicking on "Derby Database" on the menu on the left hand will open the following window:

🔼 Preferences		×
General	Derby Settings	
🖃 Derby Database	Derby Host localhost Port 1527	]
······	Memory used to start Derby Server 1024	
Eearch Engine     Ion Editor	Derby Jar Location	
	C:/org.apache.derby.core_10.2.2	
	User Define Browse	

#### Derby Host

The name of the "Derby Host" as well as the "Port" number will come up by default and can be changed if needed.

#### Memory used to start Derby Server

The amount of "Memory used to start Derby Server" will also come up by default but can be changed if more memory is available.

#### Derby Jar Location

The "Derby Jar Location" panel will list the location of the Derby Jar file by default. If you would like change this location, check the "User Define" box and click on the "Browse" button to select a new location.

Click the "Apply" button to save any changes you have made.

#### Performance

Clicking on "Performance" on the menu on the left hand will open the following window:

🔼 Preferences		
🖃 General	Performance	
GUI		
RMI Connections	Computer Performance	
Derby Database		
Performance	Single Core Dual Core Quad Core	
표 Instrument		
∃ Search Engine	Show 3D View	
Ion Settings     ■		
	Fast Loading Files	

#### Computer Performance

Select the number of cores that your computer contains (i.e. single, double or quad core). Please note that the setting of number of cores that you are able to use must comply with the license.

#### 3D view

PEAKS will display a 3D view with your quantification results. Check the "Show 3D View" box to enable this function. PEAKS 5 comes with the Java3D program to support the viewing of 3D images.

#### Fast loading files

This function is for raw file loading. If you check the "Fast Loading Files" box, PEAKS will only load spectrum header information without the peak list. Please note that if you set up a project which contains more than 20 raw files, this function will not work well due to memory issues, and you should uncheck the "Fast Loading Files" box.

Click the "Apply" button to save any changes you have made.

#### Instrument Preferences

This section will allow you to change any preferences for the following instruments: ABI, Bruker, Shimadzu and Varian.

#### ABI (.wiff)

Clicking on "Instrument" and then "ABI (.wiff)" on the menu on the left hand side will open the following window:

📐 Preferences	
⊞ General	ABI (.wiff)
Instrument	
	Default .wiff raw file convertor location
🖙 🖃 Bruker(.yep/baf, fid)	C:\Program Files\Infochromics\MSX\MSX.exe
	Browse
Varian(.xms)	
E Search Engine	
∎ Ion Editor	Raw file convertor options         ABI raw files may contain several samples, do you want to merge all the samples into one data set?         yes       Image: mail of the samples into a set in the samples inthe samples inthe samples into a set in the samples inthe

#### Default .wiff raw file convertor location

Click "Browse" to tell PEAKS the location of the Default .wiff raw file converter.

#### Raw file converter options

ABI raw files may contain several samples. By default, these samples are not merged into one data set. Select "yes" if you would like PEAKS to merge all the samples into one data set.

For PEAKS to work optimally, it is important to select if the survey spectrum or the product spectrum has been centroided.

Click the "Apply" button to save any changes you have made.

#### Bruker (.yep/baf, fid)

Clicking on "Instrument" and then "Bruker (.yep/baf, fid)" in the menu on the left hand side will open the following window:

A Preferences	
General	Bruker (.yep/baf, fid files)
Instrument  ABI(.wiff)  Bruker(.yep/baf, fid)  Shimadzu AXIMA(.rur	C. (Program Piles (Common Piles (Draker Dationik (REDATEX port (Compass/port (Exc
─────────────────────────────────────	Browse
u Ion Editor	Raw file convertor options Bruker .fid file may contain several files, do you want to merge them into one data set? yes  o no

#### Default compass file location

Click "Browse" to tell PEAKS the location of the CompassXport file converter.

#### *Raw file converter options*

Bruker .fid files may contain several samples. By default, these samples are not merged into one data set. Select "yes" if you would like PEAKS to merge all the samples into one data set.

Click the "Apply" button to save any changes you have made.

#### Shimadzu AXIMA (.run)

Clicking on "Instrument" and then "Shimadzu AXIMA (.run)" in the menu on the left hand side will open the following window:

A Preferences	
<b>⊥</b> General	Shimadzu AXIMA (.run)
🖃 Instrument	
ABI(.wiff)	Shimadzu run2xml.exe file location
Bruker(.yep/baf, fid)	
	Browse
Varian(.xms)	
Ion Editor	

Click "Browse" to tell PEAKS the location of the Shimadzu run2xml.exe file. Click the "Apply" button to save any changes you have made.

#### Varian (.xms)

Clicking on "Instrument" and then "Varian (.xms)" in the menu on the left hand side will open the following window:

A Preferences		×
T Canaval	Varian (.XMS) Default xmlrai.exe location Browse	

Click "Browse" to tell PEAKS the location of the xmlrai.exe file. Click the "Apply" button to save any changes you have made.

#### Search Engine Preferences

#### **Mascot Settings**

Clicking on "Search Engine" and then "Mascot Settings" on the menu on the left hand side will open the following window:

General     General	Mascot Settings	
🗉 Instrument		
🖃 Search Engine	Host name (or IP address)	www.matrixscience.com
Mascot Settings	Port	80
🖂 XTandem Settings	Port	
🖂 Omssa Settings	Virtual Directory	1
Sequest Settings	User name	llau
⊞ Ion Editor	Password	
	Email	llau@bioinfor.com
		Test connection Save Passwo

In this window you will tell PEAKS how to access your Mascot server (if applicable). Enter the Host name (or an IP address), Port, Virtual Directory as well as your user name, password and email address. To make sure that you entered everything correctly and that the server is working, click the "Test Connection" button. If you would like to save your password so that you don't have to enter it every time, check the "Save Password" box. Click the "Apply" button to save any changes you have made.

#### X!Tandem Settings

Clicking on "Search Engine" and then "X!Tandem Settings" in the menu on the left hand will open the following window:

📐 Preferences	
⊞ General	XTandem Settings
🗄 Instrument	
Search Engine	Launch Server     O Local Search
Mascot Settings	XTandem Server Settings
XTandem Settings	
Omssa Settings	Host name (or IP address)
	Port
🖃 Ion Editor	Fort
	Test connection
🖳 🖃 Advanced Ion Editor	
	XTandem Local Settings
	Browse

First you must select whether you would like PEAKS to access a server or local version of X!Tandem. If you select the server version, you must enter the Host name (or IP address) as well as the port. To make sure that you entered everything correctly and that the server is working, click the "Test Connection" button.

If you select a local version of X!Tandem, you must click the "Browse" button to tell PEAKS where to find the local settings. If PEAKS provides the local copy, uses the location of PEAKS as the default path. Click the "Apply" button to save any changes you have made.

#### **OMSSA Settings**

Clicking on "Search Engine" and then "OMSSA Settings" in the menu on the left hand will open the following window:

🔼 Preferences		
	Omssa Settings	
<ul> <li>Search Engine</li> <li>Mascot Settings</li> <li>XTandem Settings</li> <li>Omssa Settings</li> <li>Sequest Settings</li> <li>Ion Editor</li> </ul>	Default Omssa Path	Browse

To use OMSSA, you must click the "Browse" button to tell PEAKS where to find the default path. If PEAKS provides the local copy, uses the location of PEAKS as the default path. Click the "Apply" button to save any changes you have made.

#### **Sequest Settings**

Clicking on "Search Engine" and then "Sequest Settings" in the menu on the left hand will open the following window:

🔼 Preferences		To use
General	Sequest Settings	Sequest you
🗄 Instrument		must click
🖃 Search Engine	Sequest Location Browse	the
		"Browse"
		button to tell
Omssa Settings	Default Sequest Parameter File (.params) Browse	PEAKS
	C:\Xcalibur\params\orbi5ample.params	where to
🗉 Ion Editor		find the
	Sequest Result Output Folder Browse	default path.
	C:\Xcalibur\sequest	You must
		also browse
-		your

computer to find the location of the "Default Sequest Parameter File (.params)" as well as the "Sequest Result Output Folder". Click the "Apply" button to save any changes you have made.

Ion Editor Preferences	📐 Preferences		
Clicking on "Ion Editor" on the menu on the left hand will open the following window:	<ul> <li>              € General      </li> <li>             Instrument         </li> <li>             Search Engine         </li> <li>             Ion Settings         </li> </ul>	General Show Decimal Places: 2 2	

#### Decimal places

Select the number of decimal places you would like to appear in the ion table. The default is set to two decimal places.

#### Ion Editor

Clicking on "Ion Settings" and then "Ion Editor" in the menu on the left hand will open the following window:

📐 Preferences			
∃ General ∃ Instrument	Ion Editor		
Inscribence	Choose the ion types and the	eir charges then add them to the ion table colu	ımn list.
Ion Settings	Ion Types		Ion Table Columns
Ion Editor	Immonium a a-H2O a-NH3 b b-H2O b-NH3 c c-H2O c-H2O c-NH3 × ×-H2O ×-NH3 y y-H2O y-NH3 z z-H2O z-NH3 z z-H2O z-NH3 z	1         => Add with charge         Remove from list <=	Immonium a b b-H2O c y y-H2O z z' y (2+)
		OK Cancel	Apply

To select an ion type to be viewed in the ion table, click on the ion type in the "Ion Type" list found on the left hand side of the window. You now need to select the charge for that ion type from the drop-down menu. Once you have done this, click on the "=>Add with charge" button and the ion type will now appear in the "Ion Table Columns" list on the right hand side of the window. To remove an ion type from the "Ion Table Column" list, select the ion type and click on the "Remove from list <=" button. The ion type will now appear in the "Ion types" list. Click the "Apply" button to save any changes you have made.

# **14.2 PEAKS Configuration**

This step includes configuration of enzymes, PTMs, databases, instruments, and parameters.

To begin click the Configuration toolbar icon or Or Select "Configuration" from the "Windows" menu.

#### **Enzyme** Configuration

PEAKS can use almost any enzyme, or combination of enzymes in your analysis. You can select from any of the built-in enzymes or define your own. From the "Configuration" window select "Enzyme" from the left hand to change your enzyme configuration.

#### **Built-in enzymes**

All of the built-in enzymes within PEAKS are listed in the "Enzyme list". Clicking on one of these built-in enzymes will display the information listed about that enzyme in the "Enzyme Details" panel. Note that you cannot delete or change the details of a built-in enzyme and therefore the "Delete enzyme" button and the "Digest Rules" panel will be grayed out.

#### Create a new enzyme

Click on the "New Enzyme" button".

*Digest Rules*: This is how you specify where you enzyme will cleave the protein between two amino acids to create peptides. The letter X denotes 'any amino acid in this position', while {set brackets} indicate any amino acid except the one in the brackets. You can also choose to select the check the box "Select peptides that satisfy the above rules at both ends" if you desire.

The example below shows a combination of Trypsin and Asp-N.

🖄 Configuration		
🕗 Enzyme	Enzyme List	
D PTM	Trypsin and Asp-N combination New Enzyme	
Database	<built-in> Arg C <built-in> Arg Delete Enzyme</built-in></built-in>	í.
S. N. S. M. S.	<built-in> Asp N <built-in> Asp N + N-terminal Glu</built-in></built-in>	J
Instrument	<built-in> Chymotrypsin</built-in>	
Parameters	<built-in> CNBr <built-in> Glu C (bicarbonate)</built-in></built-in>	
	<pre><built-in> Glu C (phosphate) </built-in></pre>	
	Enzyme Details	
	Litzynie Decails	
	Enzyme Name: Trypsin and Asp-N combination	
	Digest Rules	
	Cleavage Site	
	residues at the end of a peptide KR {P} start of a new peptide	
	And/Orresidues at the end of a peptide X D start of a new peptide	
	And/Orresidues at the end of a peptide start of a new peptide	
	And/Orresidues at the end of a peptide	
	Find peptides that satisfy the above rules at both ends	
	Add/Updat:	е

You must click the "Add/Update button for the changes to be saved. Your new enzyme will now appear in the "Enzyme List" where you can access it later. If you wish to delete an enzyme that you created, select the appropriate enzyme and click the "Delete Enzyme" button.

Note: For information on defining new enzymes "on the fly" for PEAKS *de novo* or PEAKS Protein ID, see pg 45 or pg 53, respectively.

#### PTM Configuration

From the "Configuration" window select "PTM" from the left hand to change your PTM configuration.

#### **Built-in PTMs**

The built-in PTMs within PEAKS are listed in the "PTM List". To see additional built-in PTMs from the Unimod library, click the "Show unimod" box. Clicking on one of these built-in PTMs will display the information listed about that PTMs in the "PTM Details" panel. Note that you cannot delete or change the details of a built-in PTM and therefore the "Delete PTM" button and the "PTM Rules" panel will be grayed out.

#### Create a new PTM

Click on the "New PTM" button". Now simply enter the information about your PTM in the "PTM Details" panel.

Name: this name will appear in the PTM list for future use after it is saved.

Monoisotopic mass: the mass that the residue gains or loses as a result of the PTM.

*Neutral loss mass*: the mass that the modified residue loses as a result of fragmentation. Ex. 28 would signify a loss of 28 Daltons.

*Chemical formula*: the chemical formula of the PTM. This should correspond to the mass listed above.

*Residues that can be modified*: Enter residues that can be modified anywhere, residues that can only be modified if they are *at the N-or C-terminus or in the middle only*.

*Rule*: you can enter a comment for your reference.

You must click the "Add/Update button for the changes to be saved. Your new PTM will now appear in the "PTM List" where you can access it later. If you wish to delete a PTM that you created, select the appropriate PTM and click the "Delete PTM" button.

🔼 Configuration					X
Enzyme	PTM List				J
🕙 РТМ	MyCarbo Oxidation on Methionine	-)		^	New PTM
Oatabase	<built-in> 4-hydroxynonenal (HN <built-in> Acetylation (K)</built-in></built-in>	E)			Delete PTM
Instrument	<built-in> Acetylation (N-term)</built-in>				
🥙 Parameters	<built-in> Amidation <built-in> Applied Biosystems clear <built-in> Applied Biosystems clear</built-in></built-in></built-in>			~	
	Show unimod				
	PTM Details				
	PTM name:	Oxidation on Methionine			
	Mass (Monoisotopic):	15.994915			
	Neutral loss mass (Monoisotopic):	0.0			
	Residues that can be modified:	М	Anywhere 😽		
	Formula:	0			
	Rule:				
					Add/Update

The example listed below is one where we knew that only methionine was oxidized.

Note: For information on defining new PTMs "on the fly" for PEAKS *de novo* or PEAKS Protein ID, see pg 45 or pg 53, respectively.

#### Database Configuration

In addition to *de novo* sequencing of peptides, PEAKS 5 also has the ability to search through a database search to identify proteins. In order to use this function, PEAKS must have access to a protein or EST database in FASTA format or an EST database of DNA sequences. You can point PEAKS to an existing database on your system, or download one. Additionally, you can associate taxonomy with certain databases.

# WARNING: Downloading a database can take a long time (8+ hours), depending on connection speed. Most only take 20 - 30 minutes.

From the "Configuration" window select "Database" from the left hand to change your database configuration. The "Database list" at the top of the screen will show you databases that you have already configured. Select one of these files to see the details in the "Database Details" panel below.

#### Configure a new database

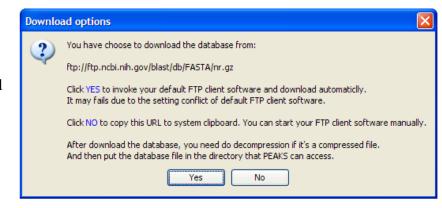
1) Select the "New Database" button on the right hand side of the "Database List". You will now be filling in the specifics for your database in the "Database Details" panel below.

ASTA format	: database:	NCBI nr			*		
Basic Options	;						
Database n	ame:	NCBI nr				Downlo	ad Database
Path:	C:\PEAKS da	tabase\NCBI nr					Browse
EST da	tabase						
Advanced Or	tions - Fasta	Title Format					
		d from FASTA title:					
\(gi\ \d*\)							
Rule to par	se description	from FASTA title:					
\s+\(.*\)							
Accession/i	d URL:						
http://www	v.ncbi.nlm.nih	gov/entrez/viewer.fcg	i?db=protein&val=	<acces< td=""><td>sion/ID&gt;</td><td></td><td></td></acces<>	sion/ID>		
Delimiter:	\s+\(.*\)			]			
axonomy O	ptions						
taxonid	I:\PEAKS dat	abase\NCBI nr\gi_taxid_	_prot.dmp.gz			Browse	Download
taxdmp 7		abase\NCBI nr\taxdmp.;			6	Browse	Download

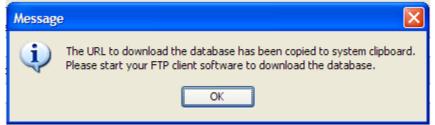
2) *Fasta Format Database*: Select your database from this drop-down menu, or select "Other" if your database is not in the list or if you would like to submit your own database.

ASTA format database:	NCBI nr	
	NCBI nr	
Basic Options	UniProtKB/Swiss-Prot	
Database name:	UniProtKB/TrEMBL	Download Database
	IPI	
Path: C:\PEAKS_	lat MSDB	Browse

3) In the basics option panel, enter a name for your database and select "Download Database".The following window will appear:



4) If you would like to invoke your default FTP client software and download automatically, click "Yes". If you select "No" the following window will appear telling you that the URL will be copied to your system clipboard. Click "Ok".



Open your Internet Explorer and paste the URL into the address bar. A file download window will open. Click Save.

5) Once the database is downloaded, you need to make sure that you decompress the file if it is compressed using a program such as WinZip, or WinRar to extract its contents. The file inside the compressed file will be a FASTA format text file (a .fas or a .fasta file).

6) Finally put the database file into a directory that PEAKS can access.

7) Click "Browse" to tell PEAKS where the database file is located.

8) If the database that you have selected is an EST database, check the box labeled "EST Database". If not, leave it blank.

9) Since you have already selected a "FASTA Format Database" in Step 2, the Accession number information and the parsing rules for the database headers are shown in the textboxes below in the "Advanced Options- Fasta Title Format" panel.

Advanced Options - Fasta Title Format
Rule to parse accession/id from FASTA title:
\(gi\ \d*\)
Rule to parse description from FASTA title:
\s+\(.*\)
Accession/id URL:
http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val= <accession id=""></accession>
Delimiter:

If you chose an "Other" (in step 2) you must enter parsing parameters yourself by typing in the textboxes. Alternatively, if our database format is the same as one of the public databases, you can choose to apply that database's format when PEAKS reads our database. Select the database that is similar to yours from the dropdown list to fill the textboxes with the appropriate parsing rules.

#### A note on parsing rules

Apart from starting with a "greater than" symbol, the precise syntax of the FASTA title line varies from database to database. For this reason, PEAKS uses Java *Regular Expressions* to define how the accession string and the description text should be parsed from the FASTA title line.

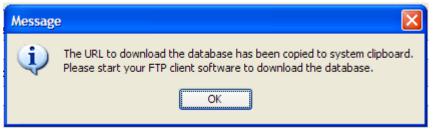
#### A note on using a delimiter

Some databases use one entry to represent multiple protein entries. The FASTA headers are concatenated with a delimiter. Since some of these databases use unprintable control codes as delimiters PEAKS will use the equivalent ASCII decimal code to represent them. For example the NCBI NR database uses CTRL-A as a delimiter so the user should input "1" as its equivalent decimal delimiter as listed here.

10) To be able to do PEAKS Protein ID using a specific taxonomy, you will need to download some files and place tell PEAKS where to find them in the "Taxonomy Options" panel.

11) To download the taxonid file, click the "Download" button. The following window will appear:





If you would like to invoke your default FTP client software and download automatically, click "Yes". If you select "No" the following window will appear telling you that the

URL will be copied to your system clipboard. Click "Ok".

Open your Internet Explorer and paste the URL into the address bar. A file download window will open. Click Save. Repeat Step 11 taxdmp file. Please not that you do not have to decompress the taxonomy files.

12) Now that you have downloaded the taxonomy files, you must tell PEAKS where to access them by clicking the Browse button and selecting the file.

13) To save the database to your "Database List", you must click the "Add/Update" button before clicking the "Ok" button.

#### Delete a previously saved database

If you would like to delete a database file, select the database that you wish to delete and click on the "Delete Database" button.

#### Set a database as default

Select the file and click the set as default button which is located to the right of the "Database List". This database will now be used by PEAKS when you run PEAKS Protein ID.

#### Moving/Updating a Database

If you choose to move a database to another directory, or delete it entirely, you need to notify PEAKS. You must remove the database from the list and re-load it. Until you do so, the database name will appear in red in the list of databases and any protein identification using that database will fail.

If you choose to update the database (perhaps by downloading the latest database file and overwriting the old database file), PEAKS will show the database information in light gray. A *light grey colour could also mean that the database does not have header information*.

#### Best practices: configuring databases for use with X!Tandem

At the time of this writing, X!Tandem had trouble searching through large databases, and would crash. It is therefore suggested that X!Tandem only be used with small databases; or if used with a large database, a taxon should be specified. The NCBInr and SwissProt databases are ideal for this purpose.

#### Best practices: configuring databases for use with OMSSA

At the time of this writing, we could not use OMSSA with databases that were not in NCBI format, or SwissProt format, and have those results available to inChorus.

Also, a bug in OMSSA prevents us from easily using databases with OMSSA when they are stored in a folder that contains a space in its path. This creates problems when PEAKS creates temporary databases on our behalf. To avoid this, best practices suggest that you put all our databases in a folder "c:\peaksdatabases". The folder "c:\my documents\databases" wouldn't work because it contains a space between 'my' and 'documents'. Using spaces in the database file name causes the same problem. So after you download and extract our database you should call the database file "ncbinr.fas", or "ncbi\_nr.fas" rather than "ncbi nr.fas".

#### Instrument Configuration

From the "Configuration" window select "Instrument" from the left hand to change your instrument configuration.

#### **Built- in Instruments**

Select the manufacturer of your instrument from the drop-down list. The names of the instruments will then appear in their vendor specific formats. Select your instrument and you will be able to view the information on your instrument in the "Instrument details" panel below. You can also select "General" in the manufacturer list and the instruments will be listed in a general format.

Note that you cannot delete or change the details of a built-in instrument and therefore the "Delete PTM" button and the "Instrument Details" panel will be grayed out.

#### Create a new instrument

Click on the "New Instrument" button and the following window will appear:

	Configuration	
In the "Instrument Details"	🕙 Enzyme	Instrument List
panel, create a name for	PTM Database	Manufacturer:General New Instrument
your instrument.	<ul> <li>Database</li> <li>Instrument</li> <li>Parameters</li> </ul>	FT-trap FT-trap (ecd-cid) FT-trap (ecd-cid) FT-trap (edd) FT-trap (ecd) FTMS FTMS (ecd)
Next, fill in your details in		FTMS (ecd-cid)
the "Basic Options" panel.		Instrument Details
In the manufacturer drop-		Instrument Name:
down list, select a specific		Basic Options Manufacturer: Aglient Technologies
vendor or "General".		Ion Source: MAIDI/SELDI
		MS Precursor Scan: 3D Ion Trap
		Fragmentation Type: CID, CAD, IRMPD (y and b ions)
		MSn Product Scan: 3D Ion Trap
		Advanced Options Precursor mass search type:  Monoiostopic Average Parent mass error tolerance:  Fragment mass error tolerance:  da
		OK Cancel Help

*Ion Source*: Use the drop-down list to select what ion source that was used; MALDI/SELDI or ESI(nano-spray). This will help the PEAKS Data Refine tool to decide the charge of the ions.

*MS- Precursor Scan*: Use the drop-down list to select what type of MS scan was performed. This selection will tell the PEAKS Data Refine tool if the survey scan is of sufficient resolution to determine the charge and the monoisotopic peak from the examination of the survey scan.

*Fragmentation type*: Use the drop-down list to select the method of fragmentation that was used. This selection will tell PEAKS what type of ion-series to expect for PEAKS auto *de novo* sequencing and PEAKS protein ID database search. Select CID/ECD if alternating fragmentation is used to allow the algorithm to determine the type of fragmentation from each scan header.

*MS<sup>n</sup> Product Scan*: Use the drop-down list to select what type of *MS<sup>n</sup>* scan was performed. This selection will help PEAKS decide which internal parameters (for weighing fragments and amount of noise) to use during PEAKS auto *de novo* sequencing and PEAKS protein ID database search. Select LIT/FT if alternating hi-res/low-res modes are used, allowing the algorithm to determine the mass analyzer from the scan header.

You can also use the "Advanced Options" to specify additional parameters.

*Precursor Mass Search Type*: Select "Monoisotopic" or "Average. For ion-trap instruments, it is usually beneficial to allow the PEAKS protein ID database search to use an average mass.

*Parent* and *Fragment error mass tolerance*: User specified values. These will appear on the PEAKS *de novo* and PEAKS protein ID options screens when the instrument is selected.

*Target Ions*: Select which ions that you would like PEAKS de novo and Protein ID to focus their search on.

You must click the "Add/Update button for the changes to be saved. Your new instrument will now appear in the "Instrument List" where you can access it later. If you wish to delete an instrument that you created, select the appropriate instrument and click the "Delete Instrument" button.

#### Parameter Configuration

From the "Configuration" window select "Parameters" from the left hand to change your parameter configurations. Please note that you can only view and delete parameters from within this parameter window. From the "Parameter type" drop-down list at the top of the screen you can select *De novo*, PEAKS Parameters or SPIDER Parameters. The parameters that you have saved within these categories will be displayed below in the list. Select the parameter file that you would like to view.

#### Creating a new parameter file

If you would like to create and save new parameters you can do this when/before you set up auto *de novo* sequencing (see page 45), PEAKS protein ID (see page 54) or SPIDER (see page 66). These references will provide you with an explanation of all of the parameters.

#### Deleting a previously saved parameter file

If you would like to delete a parameter file, select the file that you wish to delete and click on the "Delete" button.

#### Viewing a previously saved parameter file

Selecting a file will display the details of that file below. For an explanation of the parameters, please see the pages listed in the "Creating a new parameter file" section above.

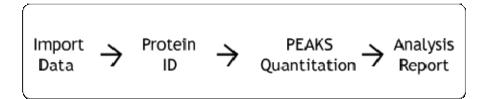
Chapter 15

# **15. PEAKS Quantification**

Many approaches to protein quantification using mass spectrometry data have been described in the literature. In terms of their implementation, most of them can be classified into three protocols.

- **MS:** Quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors within a single data set. This is the most widely used approach, which can be used with any chemistry that creates a precursor mass shift. For example, ICAT and SILAC.
- **MS/MS:** Quantification based on the relative intensities of fragment peaks at fixed m/z values within an MS/MS spectrum. For example, iTRAQ and Tandem Mass Tags.
- Label free: Label free quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors in multiple data sets aligned using mass and elution time.

All three protocols are fully implemented within PEAKS Q. The flow chart is shown below:



# 15.1 Setting up PEAKS Q Parameters

- 1) In the "Project View Frame", select a PEAKS Search result file.
- 2) Click the PEAKS quantification toolbar icon Q. Or

Select "Quantification" from the "Tools" menu.

The following window will open, displaying the quantification parameters:

Data Refine     Basic Options     Mass Error Tolerance:     Da      Upper Bound of Precursor Ch	arge: 3 🗘
. De novo	
. PEAKS Search Label Options	
. SPIDER Search   Labelling occurs at the MS/MS level eg. iTRAQ	
. PTM Finder C Label-Free	
✓ Quantification Sample Reporter Ion (Da) Labelling Efficien	icy (%)

Slightly different options will be available if you select labeling at the MS level:

🔼 Quantification		×
Tools	Quantification	
. Data Refine . De novo	Basic Options         Mass Error Tolerance:       Da          Upper Bound of Precursor Charge:       3          Retention Time Range:       min.	
. PEAKS Search	Clabel Options	
. SPIDER Search . PTM Finder	Labelling occurs at the MS/MS level eg. iTRAQ     Labelling occurs at the MS level eg. ICAT     Label-Free	
√ Quantification	Sample Added Mass Residues Labelling Efficiency (	
	Add Label Delete Label	

Quantification parameter options include the following:

#### **Basic Options**

*Mass Error Tolerance*: Quantification is based on the feature of a peptide that identifies its origin in the sample mixture. For example, in a SILAC experiment, one feature is unmodified peptides and the other is peptides modified with Label:13C(6) on arginine or lysine. For iTRAQ, the feature would be reporter ion m/z value. The mass error tolerance is for pairing up features.

*Upper Bound of Precursor Charge*: The peptide may present in different charges. Upper bound of precursor charge defines the maximum charge of peptides which are used for counting quantity.

*Retention Time Range*: The retention time range is for pairing up features. For iTRAQ, it is optional.

### **Labeling Options**

*Labeling occurs at the MS/MS level e.g. ITRAQ*: It is for quantification based on the relative intensities of fragment peaks at fixed *m/z* values within an MS/MS spectrum.

*Labeling occurs at the M level e.g. ICAT*: It is for quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors within a single data set.

*Label-free*: It is for quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors in multiple data sets aligned using mass and elution time.

Sample: It is for specifying sample name.

Reporter ion: It is for specifying mass of reporter ion

Added Mass: The modified mass of a residue.

Residues: The residue to be modified.

Labeling efficiency: It is for specifying efficiency of chemical reaction.

Add label: It is used to add a label.

Delete label: It is used to delete a label.

## 15.2 3D View

In order to produce a 3D view, you must first select this in your preferences.

1) Click on the Preferences toolbar icon  $\bigotimes$ .

or select "Preferences" from the "Windows" menu.

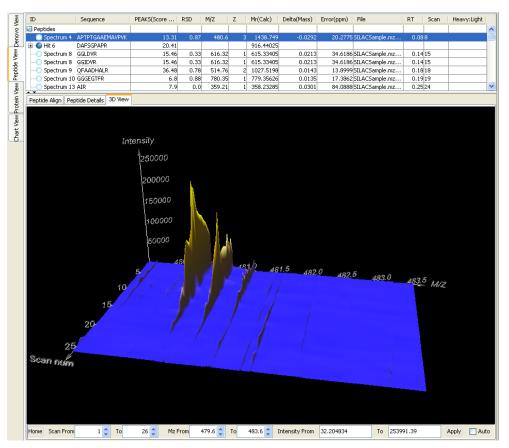
2) Select "General"
from the panel on the
left hand side.

3) Select "Performance" and check the "Show 3D View" box.

4) Click "Apply".

Preferences				<b>X</b>
🖃 General	Performance			
GUI	c			
RMI Connections	Computer Performance			
🖃 Derby Database				
Performance	Single Core	Dual Core	Quad Core	
Instrument				
∃ Search Engine	Show 3D View			
Ion Settings     ■	Fast Loading Files			
	T asc coading tiles			
				Apply
		ОК	Cancel	Help

When a PEAKS Quantification run is complete, a "3D View" tab can be found in the "Peptide View" window:



The 3D view contains 3 axes: intensity, m/z ratio and scan number.

The panel along the bottom allows you to narrow in on the peptides that you would like to examine.

You can specify a particular scan number range, m/z range or intensity range.

Click the "Apply" button to change the 3D view to your specified values.

Check the "Auto" button" and click "Apply" to the default numbers.

# 15.3 iTRAQ Walkthrough

Isobaric tagging for relative and absolute quantification (iTRAQ) uses isotopic labeling to enable relative quantitative comparisons. Up to eight different proteomic samples can be labeled using

eight different isobaric tags.

## 1) Creating a Project

Click on the "Create new project" icon or select the "New project" from the File menu. The following window will appear:

📐 New Project			
Steps	Project Properties		
1. Project Properties	Project Name:	iTRAQ Sample	
2	Project Location:	D:\derbyServer\serverDB	Browse
	Project Folder:	D:\derbyServer\serverDB\iTRAQ Sample	
	Notes/Description:		
	Type and organization	of project:	
	<ul> <li>Basic Project</li> </ul>		
	<ul> <li>Several non-labella</li> </ul>	ed samples for comparison (each sample can be fractionated	)
		< <back next="">&gt; Cance</back>	I Help

Give your project a name, such as iTRAQ Sample. Then click "Next". The following window will appear. Give your sample a name such as iTRAQ 1.

Click the "Add a file for this sample" button and select the file "C:/PEAKS 5/Data/iTRAQSample.mzx ml". Click "OK" to add this to the list of selected files.

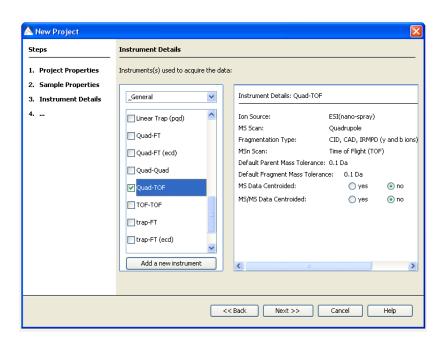
For cases where you want to add another sample to the project, select "Add another sample" and repeat these last two steps. In our case we are not going to add any more samples, so we can just click "Next".

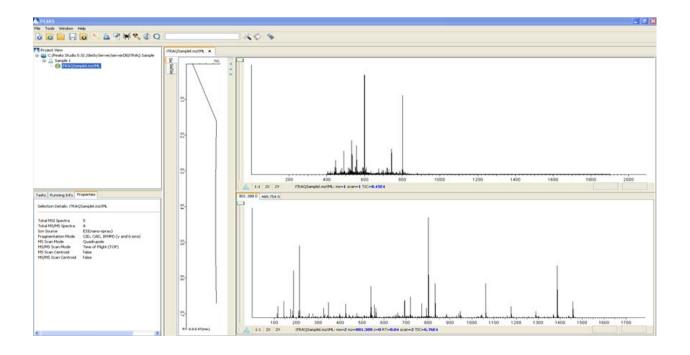
Steps 1. Project Properties	Sample Properties			
	Give this sample a name:	iTRAQ 1		~
2. Sample Properties	Select Files:			
3	Name	Size	Date Modified	Туре
	Add a file for this se Sample Notes/Description:		ove file from list ( ar sample Ren Next >> Can	Clear list

🔼 New Project		×
Steps	Sample Properties	
1. Project Properties	Give this sample a name: ITRAQ 1	~
2. Sample Properties	Select Files:	
3	Name Size Date Modified Type	
	\Sample_iTRAQ.mzXML 4,352 KB 01/22/2009 12:12:44 PM	mzxml

The following section will tell PEAKS which type of mass spectrometer was used to generate the data. This sample was derived from a Quad-TOF. Click the box beside "Quad-TOF" to select this instrument.

Upon clicking "Next" a sample project will be created. Your "Main Processing Screen" should look something like this:





# 2) Running data refinement

In the "Project View Frame", select iTRAQSample.mzxml.

Click the data refinement tool  $\checkmark$  or select "Data Refine" from the "Tools" menu. Enter the following parameters shown below and click "OK".

🖄 Data Refine		
Tools	Data Refine	
✓ Data Refine	Merge Options Merge scans of the same peptide:	⊖ yes ⊙ no
. De novo	Retention time window: (for raw files only)	min.
. PEAKS Search	m/z tolerance:	Da 💌
. SPIDER Search	Charge Options	
, PTM Finder	Correct precursor charges: Minimum charge: 1 Ma	• yes O no
. Quantification		
	Filter Options	
	Filter MS/MS scans:	O yes ⊙ no
	Precursor mass between	and Da
	Retention time between	and min
	Quality value greater than	suggest 0.65
	Preprocess Options	
	Preprocess MS/MS scans:	🔿 no, already done 💿 yes 🔿 no
		OK Cancel Help

# 3) Running PEAKS Search

Click the PEAKS Search toolbar icon or select "PEAKS Search" from the Tools menu.

The Protein Identification Parameters dialogue window will appear. Input the parameters as following.

A PEAKS Search	
Tools	Database Search Save Parameter ITRAQ
. Data Refine . De novo ✓ PEAKS Search . SPIDER Search . PTM Finder	Mass Options       General Options         Parent Mass Error Tolerance:       0.3       Da       Precursor Mass Search Type:       Preprocess this data 'on the fly'         Fragment Mass Error Tolerance:       0.1       Da       Monoisotopic       Average         Enzyme Options       Image: Comparison of the fly of the statisfy the above rule at both ends       New Enzyme       Image: Comparison of the fly of the statisfy the above rule at both ends
, Quantification	PTM Options         Name       Mono mass       Residue site       Add Fixed =>       Fixed Modification         Acetylation (N 42.0106       KI       Add Pixed =>       Applied Biosystems (TRAQ(TM) 4plex (N)         Acetylation (N + 42.0106       KI       Image: Applied Biosystem (TRAQ(TM) 4plex (N)       Applied Biosystems (TRAQ(TM) 4plex (N)         Applied Biosyst 450.2752       CI       Image: Add Variable =>       Variable Modification       Image: Oxidation M         Applied Biosyst 236.1572       CI       Image: Add Variable =>       Variable Modification       Image: Oxidation M         Applied Biosyst 236.1572       CI       Image: Add Variable =>       Variable Modification       Image: Oxidation M         Applied Biosyst 236.1572       CI       Image: Add Variable =>       Variable PTM per peptide:       Image: Oxidation M         Applied Biosyst 236.1572       CI       Image: Add Variable =>       Max variable PTM per peptide:       Image: Oxidation M         Show unimod       New PTM       Max variable PTM per peptide:       Image: Oxidation M       Image: Oxidation M         Image: Oxidation State       Select Database:       Swissprot       Image: Oxidation M       Image: Oxidation M       Image: Oxidation M         Image: Oxidation State       Select Databases:       Swissprot
	Advanced Options PEAKS uses a hybrid search technique that requires some sequence tags to help in the search I have already run de novo, don't run it again Run de novo using different parameters than the above ORun de novo using the same parameters as above (default) Validation - decoy search OK Cancel Help

Click "OK". This will launch PEAKS Protein ID and when completed the results will appear, as below:

■ prodes         9         0         00         88         2         1500.6175         00.65         35.07265         sample_iTLAQ_m2041         0.042           Spectrum 3         Y[1]MYNWQH(2]GGL         1.75         0.65         681.704         3         2042.0037         -0.0864         42.3222[sample_iTRAQ_m2041         0.255           Spectrum 5         Y[1]MYNWQH(2]GGL         1.75         0.68         681.704         3         2042.0037         -0.0864         42.3222[sample_iTRAQ_m2041         0.255           Spectrum 7         M[1]SGIRR         49.87         0.01         427.8687         2         679.5297         0.0325         38.9196/sample_iTRAQ.m2041         0.255           Spectrum 7         M[1]SGIRR         49.87         0.01         427.727         2         853.46027         0.0208         24.3872[sample_iTRAQ.m2041         0.5911           Spectrum 8         V[1]DEDQFPAVFK[2]         76.95         0.71         815.43         1         1407[sample_iTRAQ.m2041         0.5911           Spectrum 8         V[1]DEDQFPAVFK[2]         76.95         0.71         815.43         1         1407[sample_iTRAQ.m2041         0.5911           Spectrum 8         V[1]DEDQFPAVFK[2]         76.95         0.71         11.25 <td< th=""><th>Spectrum         Y[1]MNWQ4{Z}[2]GL         1.75         0.65         681.764         3         2042.0037         -0.0664         42.32225angle_IT8AQ,mc2ML         0.0.42           Spectrum         Y[1]MYNWQ4{Z}[2]GL         1.75         0.65         681.704         3         2042.0037         -0.0664         42.32225angle_IT8AQ,mc2ML         0.225           Spectrum         D[1]MENPGVTQLNR         97.81         0.15         766.867         1571.7815         0.022         13.97975angle_ITRAQ,mc2ML         0.5916           Spectrum         M[1]SGLFR         48.87         0.01         427.727         2         853.46027         0.0208         24.33725angle_ITRAQ,mc2ML         0.5911           Spectrum         M[1]SGLFR         48.87         0.01         427.727         2         853.46027         0.0208         24.33725angle_ITRAQ,mc2ML         0.5911           Spectrum         M[1]SGLFR         48.87         0.01         427.727         2         853.46027         0.0208         24.33725angle_ITRAQ,mc2ML         0.7613           Peptide Declasis         Timmonum         b         b         142.372         353.2317         141.102070pA         1396.66         1390.66         1390.66         1390.66         1390.66         1390.66         1390.46</th></td<> <th>Spectrum         1 A(1) EXN016/05EAT         99.0         0.0         001.388         2         1600.8176         0.06652         35.07265         35.0726         37.0726         35.0721         35.0726         35.0721         35.0726         35.0721         35.0726         35.0721         35.0726         35.0721<!--</th--><th>■ Spectrum         1 A(1)ENND(05%5A1R         99.0         0.0         001.888         2         1600.8176         0.0562         35.0785 Sample_ITRAQ.mc2ML         0.0.42           Spectrum         Y[1]MYNWQHK[2]3GL         1.75         0.65         681.704         3         2042.0037         -0.0864         42.32225         58.9186_BTRAQ.mc2ML         0.225           Spectrum         5 (1)IMENRGVTQLNR         97.81         0.15         786.887         1571.7815         0.002         13.9797/Sample_ITRAQ.mc2ML         0.521           Spectrum         7 (1)IMENRGVTQLNR         97.81         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.591111           Spectrum</th><th>ID</th><th>Seq</th><th>Jence</th><th>PEAKS(</th><th>Score %)</th><th>RSD</th><th>M/Z</th><th>Z</th><th>Mr(Calc)</th><th>Delta(Mass)</th><th>Error(ppm)</th><th>File</th><th>RT</th><th>Scan</th></th>	Spectrum         Y[1]MNWQ4{Z}[2]GL         1.75         0.65         681.764         3         2042.0037         -0.0664         42.32225angle_IT8AQ,mc2ML         0.0.42           Spectrum         Y[1]MYNWQ4{Z}[2]GL         1.75         0.65         681.704         3         2042.0037         -0.0664         42.32225angle_IT8AQ,mc2ML         0.225           Spectrum         D[1]MENPGVTQLNR         97.81         0.15         766.867         1571.7815         0.022         13.97975angle_ITRAQ,mc2ML         0.5916           Spectrum         M[1]SGLFR         48.87         0.01         427.727         2         853.46027         0.0208         24.33725angle_ITRAQ,mc2ML         0.5911           Spectrum         M[1]SGLFR         48.87         0.01         427.727         2         853.46027         0.0208         24.33725angle_ITRAQ,mc2ML         0.5911           Spectrum         M[1]SGLFR         48.87         0.01         427.727         2         853.46027         0.0208         24.33725angle_ITRAQ,mc2ML         0.7613           Peptide Declasis         Timmonum         b         b         142.372         353.2317         141.102070pA         1396.66         1390.66         1390.66         1390.66         1390.66         1390.66         1390.46	Spectrum         1 A(1) EXN016/05EAT         99.0         0.0         001.388         2         1600.8176         0.06652         35.07265         35.0726         37.0726         35.0721         35.0726         35.0721         35.0726         35.0721         35.0726         35.0721         35.0726         35.0721 </th <th>■ Spectrum         1 A(1)ENND(05%5A1R         99.0         0.0         001.888         2         1600.8176         0.0562         35.0785 Sample_ITRAQ.mc2ML         0.0.42           Spectrum         Y[1]MYNWQHK[2]3GL         1.75         0.65         681.704         3         2042.0037         -0.0864         42.32225         58.9186_BTRAQ.mc2ML         0.225           Spectrum         5 (1)IMENRGVTQLNR         97.81         0.15         786.887         1571.7815         0.002         13.9797/Sample_ITRAQ.mc2ML         0.521           Spectrum         7 (1)IMENRGVTQLNR         97.81         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.591111           Spectrum</th> <th>ID</th> <th>Seq</th> <th>Jence</th> <th>PEAKS(</th> <th>Score %)</th> <th>RSD</th> <th>M/Z</th> <th>Z</th> <th>Mr(Calc)</th> <th>Delta(Mass)</th> <th>Error(ppm)</th> <th>File</th> <th>RT</th> <th>Scan</th>	■ Spectrum         1 A(1)ENND(05%5A1R         99.0         0.0         001.888         2         1600.8176         0.0562         35.0785 Sample_ITRAQ.mc2ML         0.0.42           Spectrum         Y[1]MYNWQHK[2]3GL         1.75         0.65         681.704         3         2042.0037         -0.0864         42.32225         58.9186_BTRAQ.mc2ML         0.225           Spectrum         5 (1)IMENRGVTQLNR         97.81         0.15         786.887         1571.7815         0.002         13.9797/Sample_ITRAQ.mc2ML         0.521           Spectrum         7 (1)IMENRGVTQLNR         97.81         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.59111           Spectrum         Y (1)ISGAFR         48.87         0.01         427.727         2         853.46027         0.0208         24.3372/Sample_ITRAQ.mc2ML         0.591111           Spectrum	ID	Seq	Jence	PEAKS(	Score %)	RSD	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(ppm)	File	RT	Scan
Spectrum 3         Y[1]MYWWQHK[2]GGL         1.75         0.65         681.704         3         2042.032         -0.0864         42.3222Sample_TRAQ.m2XM         0.2256           Spectrum +         T[1]LPISR         65.43         0.0         440.756         2         679.52997         0.0025         36.9196[sample_TRAQ.m2XM         0.226           Spectrum 7         M[1]SGIFR         48.87         0.01         427.627         2         853.46027         0.0208         24.3872[Sample_TRAQ.m2XM         0.5911           Spectrum 7         M[1]SGIFR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872[Sample_TRAQ.m2XM         0.5911           Spectrum 8         V[1]DEDQFPFAVPK[2]         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407[Sample_TRAQ.m2XM         0.76]13           Peptide Details	■         ■	Spectrum 3         YLIPWWWWE(2)G         1.7.5         0.45         81.704         3         2042.0037         -0.0864         42.3222(semble_ITRA0.mc2ML         0.225           Spectrum 5         0(1)WEMPKVTQLNR         97.81         0.15         786.887         1571.7815         0.0225         36.9196/semple_ITRA0.mc2ML         0.225           Spectrum 5         0(1)WEMPKVTQLNR         97.81         0.15         786.887         1571.7815         0.022         13.9797 Sample_ITRA0.mc2ML         0.428           Spectrum 5         V[1)BEDQFFPAVFK[2]         76.95         0.01         427.722         2653.46027         0.00208         2.3872/Sample_ITRA0.mc2ML         0.428           Spectrum 5         V[1)BEDQFFPAVFK[2]         76.95         0.71         815.43         1 1628.8652         0.0198         12.1407/Sample_ITRA0.mc2ML         0.76/13           Peptide Alon         Peptide Details         1         118.15         216.15         198.14         188.16         233.17         144.100769A         1272.61         1272.61         1272.61         1272.61         1272.61         1272.61         1272.61         1272.61         1272.61         1272.61         1272.61         1272.61         165.52         1272.61         165.52         1272.61         165.52 <td< th=""><th>Perchan 3         Y[1]/PHXWORP(E[2]G2         1.7.5         0.65         681.704         3         2042.0037         -0.0654         42.32223sende_ITRAQ.mc20ML         0.2256           Spectrum 5         0[1]/PEISR         665.43         0.0         40.756         2         579.52997         0.00325         36.9196[sample_ITRAQ.mc20ML         0.226           Spectrum 5         0[1]/PEISR         665.43         0.0         427.727         2         653.46027         0.0028         24.3872[sample_ITRAQ.mc20ML         0.428           Spectrum 7         M[1]SGIRR         48.87         0.0         427.727         2         653.46027         0.0028         24.3872[sample_ITRAQ.mc20ML         0.428           Spectrum 8         V[1]DEDQ#FPAVFK[2]         76.95         0.71         815.43         2         1628.6652         0.0198         12.1407[sample_ITRAQ.mc20ML         0.7613           Peptide Details         ///         ///         144.1007[sample_ITRAQ.mc20ML         0.721         815.43         1         1272.61         145.52         1272.61         1472.61         1472.61         1472.61         1472.61         1472.61         1472.61         1472.61         1472.61         145.52         1272.61         1472.61         1165.52         1272.61         147</th><th>😺 Peptid</th><th>les</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></td<>	Perchan 3         Y[1]/PHXWORP(E[2]G2         1.7.5         0.65         681.704         3         2042.0037         -0.0654         42.32223sende_ITRAQ.mc20ML         0.2256           Spectrum 5         0[1]/PEISR         665.43         0.0         40.756         2         579.52997         0.00325         36.9196[sample_ITRAQ.mc20ML         0.226           Spectrum 5         0[1]/PEISR         665.43         0.0         427.727         2         653.46027         0.0028         24.3872[sample_ITRAQ.mc20ML         0.428           Spectrum 7         M[1]SGIRR         48.87         0.0         427.727         2         653.46027         0.0028         24.3872[sample_ITRAQ.mc20ML         0.428           Spectrum 8         V[1]DEDQ#FPAVFK[2]         76.95         0.71         815.43         2         1628.6652         0.0198         12.1407[sample_ITRAQ.mc20ML         0.7613           Peptide Details         ///         ///         144.1007[sample_ITRAQ.mc20ML         0.721         815.43         1         1272.61         145.52         1272.61         1472.61         1472.61         1472.61         1472.61         1472.61         1472.61         1472.61         1472.61         145.52         1272.61         1472.61         1165.52         1272.61         147	😺 Peptid	les												
Spectrum 4         T[1]EFISR         65.43         0.0         440.756         2         879.52997         0.0325         36.9196/Sample_TRAQ.m2XM         0.22/6           Spectrum 5         D[1]MENFQVTQLNR         97.81         0.15         766.867         2         1571.7815         0.022         13.97975ample_TRAQ.m2XM         0.42/8           Spectrum 7         M[1]SGLR         448.87         0.0         427.727         2         853.46027         0.00208         24.3872/Sample_TRAQ.m2XM         0.5911           Spectrum 7         M[1]SGLR         448.87         0.0         427.727         2         853.46027         0.00208         24.3872/Sample_TRAQ.m2XM         0.5911           Spectrum 7         M[1]DEDQPFPAVPK[2]         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407/Sample_TRAQ.m2XM         0.76[13           Petide Align         Petide Details           1         185.43         2         1628.8652         0.0198         12.1407/Sample_TRAQ.m2XM         0.76[13           3         66.10         426.30         408.27         398.29         443.31         L         1286.66         1370.66         6593.84         13           3         66.10	■         ■         ●	■         ■         ■         ●	■ Spectrum 4       TLI_IFISR       -65.43       0.0       440.756       2       879.52997       0.0325       36.9196/Sample_TRAQ.mc2ML       0.256         Spectrum 7       M[1]SGIR       48.67       0.0       427.727       2       853.46027       0.0208       24.3872/Sample_TRAQ.mc2ML       0.59111         Spectrum 7       M[1]SGIR       48.67       0.0       427.727       2       853.46027       0.0208       24.3872/Sample_TRAQ.mc2ML       0.59111         Spectrum 8       V[1]DEGLFRA       48.67       0.0       427.727       2       853.46027       0.0208       24.3872/Sample_TRAQ.mc2ML       0.59111         Spectrum 8       V[1]DEGLFRA       48.67       0.0       427.727       2       853.46027       0.0208       24.3872/Sample_TRAQ.mc2ML       0.59111         Spectrum 8       V[1]DEGLFRA       48.67       0.01       427.727       2       853.46027       0.0208       24.3872/Sample_TRAQ.mc2ML       0.7613         Peptide Align       Peptide Align       Passed       V[1]DEGLFRA       48.67       0.71       417.727       2       853.46027       0.0208       24.3872/Sample_TRAQ.mc2ML       0.7613         Peptide Align       Peptide Align       Passed       0.200       2       40.751 </td <td></td>														
Spectrum 5         O[1]WEINPGVTQLNR         97.81         0.15         786.887         2         1571.7815         0.022         13.97975ample_TRAQ.m2ML         0.428           Spectrum 7         M[1]SGIFR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872[Sample_TRAQ.m2ML         0.59111           Spectrum 3         V[1]DEDQFPFAVPK[2]         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407[Sample_TRAQ.m2ML         0.59111           Peptide Align         Peptide Details           76         93.71         141.10207@A          12.1407[Sample_TRAQ.m2ML         0.7613           Peptide Align         Peptide Details           2         70.07         313.20         295.19         285.20         330.23         P         1366.66         1369.66         1369.66         1370.66         693.84         14           2         70.07         313.20         295.19         285.20         330.23         P         1366.66         1369.66         1369.65         1370.66         693.84         13           3         86.10         426.30         408.27         398.29         144.10207@A         110.55	Spectrum 5         D[1]MENPGYTQLNR         97.81         0.15         766.887         2         1571.7815         0.022         13.97975smple_ITRA0,mc2ML         0.428           Spectrum 7         M[1]SGIFR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872[Sample_ITRA0,mc2ML         0.5911           Spectrum 7         M[1]SGIFR         48.87         0.0         427.7272         2         853.46027         0.0208         24.3872[Sample_ITRA0,mc2ML         0.5911           Spectrum 8         V[1]DEDCPFAYPK[2]         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407[Sample_ITRA0,mc2ML         0.5911           Peptide Alion         Peptide Details          C         Seq         y         y+H2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.15         233.17         144.10207@A         z         z'         y(2+)         #           2         70.07         313.20         295.20         300.23         P         1386.65         1271.61         1370.66         645.32         14           2         70.07         313.20         295.31	■         ■	■         ■				L										
Spectrum 7         M[1]SGLFR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872[Sample_TRAQ.m2XML         0.5911           Spectrum 7         M[1]SGLFR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872[Sample_TRAQ.m2XML         0.5911           Spectrum 7         M[1]SGLFR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872[Sample_TRAQ.m2XML         0.5911           Spectrum 8         V[1]DEQDePRAPK[2]         76.95         0.71         815.43         2         1628.652         0.0198         12.1407[Sample_TRAQ.m2XML         0.76]13           Peltide Alion         Peptide Details         mmonium         b         b-H2O         a         c         Seq         y         yH2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         186.16         233.17         144.10207@A         1276.61         1370.66         693.84         13           3         86.10         426.50         400.827         398.29         443.31         L         1298.66         1366.66         1370.66         693.84         13	Spectrum 7         MLISGIFR         48.87         0.0         427.727         2         653.46027         0.0208         24.3872/smple_TRA0,m22ML         0.5911           Spectrum 7         MLISGLFR         48.87         0.0         427.727         2         653.46027         0.0208         24.3872/smple_TRA0,m22ML         0.5911           Spectrum 7         MLISGLFR         48.87         0.0         427.727         2         653.46027         0.0208         24.3872/smple_TRA0,m22ML         0.5911           Spectrum 7         MLISGLFR         48.87         0.0         427.727         2         653.46027         0.0208         24.3872/smple_TRA0,m22ML         0.5911           Spectrum 7         MLISGLFR         48.87         0.0         427.727         2         653.46027         0.0208         24.3872/smple_TRA0,m22ML         0.5911           Petide Align         Petide Details         1         108.16         233.17         144.10207@A         2         2         2         9         9         143.3         8         108.66         1369.66         1369.66         1369.66         1370.66         693.84         13           3         86.10         426.33         408.27         362.33         553.36         373.46	Spectrum 7         PMI_ISGERE         448.87         0.0         427.727         2         853.46027         0.0208         24.3872/Sample_TRAQ.mc2ML         0.5911           Spectrum 8         VI/ISCGERFAUMR(2)         76.95         0.71         815.43         2         1528.6622         0.0208         24.3872/Sample_TRAQ.mc2ML         0.59111           Spectrum 8         VI/ISCGERFAUMR(2)         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407/Sample_TRAQ.mc2ML         0.7613           Peptide Align         Peptide Align         Peptide Align         2         2         2         y         y+H2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         14         14           2         70.07         313.30         295.19         285.34         D         1176.55         1159.54         1159.52         188.75         112           4         68.04         54.12         52.30         513.26         672.38         N         1061.52         104.50         1055.56         101.56         1055.56         101.56         101.56         104.50         104.50 <td< td=""><td>Spectrum 7         PMI_ISGER         448.87         0.0         427.727         2         853.46027         0.0208         24.3872/Sample_TRAQ.mc2ML         0.59111           Spectrum 8         VI_DECQ/FPAUMYL(2)         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407/Sample_TRAQ.mc2ML         0.59111           Peptide Align         Peptide Align         Peptide Align         2         523.46027         0.0208         24.3872/Sample_TRAQ.mc2ML         0.59111           Peptide Align         Peptide Align         Peptide Align         2         523.46027         0.0208         24.3872/Sample_TRAQ.mc2ML         0.59111           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         1         14           2         70.07         313.02         259.19         285.20         9         1386.69         1566.66         1369.66         1370.66         693.84         13           4         68.04         541.22         22.30         513.32         558.34         D         1176.55         1154.41         1195.52         1105.55         588.77         11           5         67.06         655.35         637.34         627.36</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>· · - ·</td><td></td><td></td></td<>	Spectrum 7         PMI_ISGER         448.87         0.0         427.727         2         853.46027         0.0208         24.3872/Sample_TRAQ.mc2ML         0.59111           Spectrum 8         VI_DECQ/FPAUMYL(2)         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407/Sample_TRAQ.mc2ML         0.59111           Peptide Align         Peptide Align         Peptide Align         2         523.46027         0.0208         24.3872/Sample_TRAQ.mc2ML         0.59111           Peptide Align         Peptide Align         Peptide Align         2         523.46027         0.0208         24.3872/Sample_TRAQ.mc2ML         0.59111           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         1         14           2         70.07         313.02         259.19         285.20         9         1386.69         1566.66         1369.66         1370.66         693.84         13           4         68.04         541.22         22.30         513.32         558.34         D         1176.55         1154.41         1195.52         1105.55         588.77         11           5         67.06         655.35         637.34         627.36												· · - ·		
Spectrum 7         M[1]SGLFR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872         Sample_ITRAQ.m2XML         0.59         11           Spectrum 8         V[1]DEDQPFPAVPK[2]         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407/Sample_ITRAQ.m2XML         0.59         11           Peptide Align         Peptide Details         V         V=120         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A           144         1277.61         693.84         13           3         86.10         426.30         408.27         398.29         443.31         L         1289.65         1271.62         1272.61         1273.61         645.32         12           4         88.04         541.32         523.30         513.32         558.34         D         1176.55         1195.52         1160.52         588.77         11           5         87.06         655.38         657.34         627.36         672.36         71.33         742.39         787.41         D         947.48         929.47	Spectrum 7         M[1]SGLR         48.87         0.0         427.727         2         853.46027         0.0208         24.3872         Sample_TRAQ.m22ML         0.59         11           Peptide Align         Peptide Details         International and the second secon	Spectrum 7         MiljSGER         48.87         0.0         427.727         2         853.46027         0.0208         24.3872         Sample_TRAQ.m2XML         0.5911           Peptide Algn         Peptide Details         1         8         V11DEQPFPAVPK[2]         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407         5mple_TRAQ.m2XML         0.76[13           Peptide Algn         Peptide Details           V1202         z         z'         y(2+)         #           1         188.15         216.15         198.14         186.16         233.17         144.10207@A         1086.69         1369.66         1370.66         693.84         13           3         86.10         426.30         409.27         992.92         124.81         124.81         144.83         124.81         124.81         144.83         1045.55         1163.54         1045.50         533.42         583.37         141.1289.65         1369.66         1370.66         693.84         13           4         98.04         541.32         523.30         513.32         558.34         D         1176.55         1185.54         1045.50         531.26         10           6 </td <td>Spectrum 7         MiljSGER         48.67         0.0         427.727         2         653.46027         0.0208         24.3872         Sample_TRAQ.m2XML         0.5911           Peptide Align         Peptide Details        </td> <td></td> <td>_</td>	Spectrum 7         MiljSGER         48.67         0.0         427.727         2         653.46027         0.0208         24.3872         Sample_TRAQ.m2XML         0.5911           Peptide Align         Peptide Details														_
Spectrum 8         V[1]DEDQPFFAVPK[2]         76.95         0.71         815.43         2         1628.8652         0.0198         12.1407[Sample_TRAQ.m2XML         0.76[13]           Peptide Align         Peptide Details         Immonium         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         144         143         144.10207@A         144         143         1271.62         1272.61         1273.61         645.32         12         14         88.04         133.20         528.34         D         1176.55         1158.54         1159.52         1160.52         588.77         11           5         87.06         655.35         637.34         627.36         672.38         N         1061.52         1043.50         1045.50         531.20         150         152         153.51         144.16.30         1445.50         1531.50         150.51         150.51         150.51         150.51         150.51         150.51         150.51         150.51         150.51         160.31         160.41         146.73         8         30.03         <	Peptide Align         Peptide Details           Immonium         b         b-H2O         a         c         Seq.         y         yH2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A           1         149.666         1370.66         693.84         13           3         66.10         426.30         406.27         398.29         443.31         L         1271.62         1272.61         1273.61         645.32         12           4         68.04         541.32         523.34         D         1176.55         1158.54         1169.52         1160.52         583.77         11           5         67.06         655.35         637.34         627.36         672.38         N         1061.52         104.51         104.55         513.64         116           6         88.04         770.39         787.41         D         947.48         929.47         930.45         951.45         10           7         66.10         83.0.46         865.45         855.47         90.41         951.43         864.63         454.32         316.7	Spectrum 8         V[1]DEDQPFPAVPK[2]         76.95         0.71         815.43         21         1628.8652         0.0198         12.1407[Sample]_TRAQ.m2ML         0.76[3]           Peptide Alon         Peptide Details         Immonium         b         bH20         c         Seed         y         y-H20         z         z'         y(2+)         #           1         188.15         21.515         198.14         188.15         233.17         144.10207@A         144.102.07@A         115.51         159.32         153.32         153.32         153.32         155.33         153.32         155.33         155.33         155.33         155.33         155.31         159.51         159.51         159.55         1159.55         1159.55         1159.55         150.55         127.66         1273.61         147.45         144.45         145.43         145.73         8           3.0.03         940.49         922.48         957.51         G         719.38	Spectrum 8         V[1]DEDQPFPAVPR[2]         76.95         0.71         815.43         2         1628.9652         0.0198         12.1407[Sample_JTRAQ.m2ML         0.76[1]           Peptide Alon         Peptide Details         Immonium         b         bH20         a         c         Seq         y         Y+20         z         z'         y (2+)         #           1         188.15         21.515         198.14         188.16         233.17         144.10207@A         144         133         66.10         1366.66         1369.66         1370.66         693.84         133           3         86.10         426.30         408.27         398.29         443.31         L         1289.65         1271.62         1272.61         1272.61         653.27         583.37         104.351         1045.50         1045.50         583.27         11           5         87.06         655.35         637.34         627.36         672.38         N         1061.52         1043.51         1045.50         513.26         10           6         68.04         770.39         782.33         752.38         N         1061.52         1045.50         503.126         10           7         86.10         883														
Peptide Allon         Peptide Details           #         Immonium         b         b+H2O         a         c         Seq         y         y+H2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         -         -         14           2         70.07         313.20         295.19         285.20         330.23         P         1386.69         1366.66         1369.66         1370.66         693.84         13           3         86.10         426.30         408.27         398.99         443.31         L         1273.61         1273.61         645.32         12         12           4         88.04         541.32         253.30         513.32         558.34         D         1176.55         1158.54         1159.52         1160.52         583.77         11           5         67.06         655.35         637.34         627.36         672.38         N         1061.52         1043.51         1044.50         1045.50         531.26         10           6         88.04         770.39         752.37         742.39         787.41         D	Peptide Align         Peptide Details           #         Immonium         b         b+t20         a         c         Seq         y         y+t20         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         -<	Peptide Align         Peptide Details           #         Immonium         b         b-H20         a         c         Seq         y         y-H20         z         z'         y (2+)         #           1         188.15         216.15         199.14         188.16         233.17         144.10207@A         1386.69         1366.69         1369.66         1370.66         693.84         14           2         70.07         313.20         295.19         285.20         330.23         P         1386.69         1366.69         1369.66         1370.66         693.84         12           3         86.10         426.30         408.27         398.29         443.31         L         1289.65         1271.62         1273.61         645.32         12           4         88.04         541.32         523.37         367.23         N         1061.55         1158.54         1159.52         1160.52         588.77         11         D         947.48         929.47         930.45         931.45         474.24         9           7         66.10         883.46         865.45         855.47         900.49         1         832.47         814.44         816.43         416.73 <t< td=""><td>Peptide Align         Peptide Details           #         Immonlum         b         b-H20         a         c         Seq         y         y-H20         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         1386.69         1366.66         1369.66         1370.66         693.84         14           2         70.07         313.20         295.19         285.20         30.23         P         1386.69         1366.61         1370.66         693.84         12           3         86.10         426.20         408.27         398.29         143.31         L         1289.65         1271.62         1272.61         1273.61         645.32         12         12         655.55         537.34         627.39         N         1061.52         104.50         1045.50         1531.26         100           6         88.04         770.39         752.37         742.39         787.41         D         947.48         929.47         930.45         931.45         474.24         9           7         66.10         803.04         865.45         855.47         900.49         1032.1         <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>_</td></td<></td></t<>	Peptide Align         Peptide Details           #         Immonlum         b         b-H20         a         c         Seq         y         y-H20         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         1386.69         1366.66         1369.66         1370.66         693.84         14           2         70.07         313.20         295.19         285.20         30.23         P         1386.69         1366.61         1370.66         693.84         12           3         86.10         426.20         408.27         398.29         143.31         L         1289.65         1271.62         1272.61         1273.61         645.32         12         12         655.55         537.34         627.39         N         1061.52         104.50         1045.50         1531.26         100           6         88.04         770.39         752.37         742.39         787.41         D         947.48         929.47         930.45         931.45         474.24         9           7         66.10         803.04         865.45         855.47         900.49         1032.1 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>_</td></td<>														_
#         Immonium         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A         14           2         70.07         313.20         295.19         285.20         330.23         P         1386.69         1369.66         1370.66         693.84         13           3         86.10         426.30         408.27         398.29         443.31         L         1289.65         1271.62         1272.61         1655.35         645.32         12           4         88.04         541.32         523.30         513.32         558.34         D         1176.55         1158.54         1150.52         588.77         11           5         87.06         655.33         662.35         672.38         N         1061.52         1043.51         1044.50         1045.50         531.26         10           6         88.04         770.39         752.37         742.39         787.41         D         947.48         929.47         930.45         931.45         447.24         9           7	#         Immonium         b         b+l20         a         c         Seq         y         y+l20         z         z'         y(2+)         #           1         188.15         216.15         196.14         188.16         233.17         144.10207@A         144         144         127.61         1370.66         693.84         13           3         88.10         426.50         408.27         399.29         443.31         L         1289.65         1272.61         1272.61         1655.2         588.77         11           5         87.06         6555.35         637.34         627.36         672.38         N         1061.52         1045.50         1045.50         551.26         51.26         10           6         88.04         541.32         523.37         742.39         787.41         D         947.48         929.47         931.45         747.42         9           7         86.10         883.46         865.45         655.47         900.49         I         832.47         814.44         815.43         816.43         416.73         81.87           9         72.08         109.955         101.56         1055.58         Y         62.35         644.34<	#         Immonlum         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y(2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A           1         1366.66         1366.66         1369.66         1369.66         693.84         14           2         70.07         313.20         295.19         285.20         330.23         P         1366.69         1368.66         1369.66         1369.66         693.84         14           3         366.10         426.30         408.27         398.29         443.31         L         1299.65         1127.62         1127.61         1273.61         645.32         12           4         88.04         541.32         523.30         513.32         558.34         D         1176.55         1158.54         1159.52         1160.52         598.77         111           5         67.06         655.35         657.37         747.39         757.51         G         719.38         701.36         702.34         154.44.24         9           7         96.10         863.96         365.45         855	#         Immonlum         b         b-H2O         a         c         Seq         y         y-H2O         z         z'         y (2+)         #           1         188.15         216.15         198.14         188.16         233.17         144.10207@A              6         693.84         14           2         70.07         313.20         295.19         285.20         330.23         P         1366.66         1369.66         1370.66         693.84         14           3         366.10         426.30         400.827         398.29         443.31         L         1289.65         1370.66         109.52         160.52         588.77         11           5         67.06         655.35         637.34         627.36         672.38         N         1061.52         1045.50         531.26         1045.50         531.26         1045.20         193.45         474.24         9           7         66.10         883.46         855.45         855.47         990.49         I         832.47         814.44         815.43         816.43         416.73         8           8         30.03         90.49         922.48 </td <td></td> <td>bectrum 8 V[1]D</td> <td>EDQPFPAVPK[2</td> <td></td> <td>76.95</td> <td>0.71</td> <td>815.43</td> <td>2</td> <td>1628.8652</td> <td>0.0198</td> <td>12.1407</td> <td>' Sample_iTRAQ</td> <td>.mzXML  0.7</td> <td>613</td>		bectrum 8 V[1]D	EDQPFPAVPK[2		76.95	0.71	815.43	2	1628.8652	0.0198	12.1407	' Sample_iTRAQ	.mzXML  0.7	613
1         188.15         216.15         198.14         188.16         233.17         144.10207@A         P         1386.69         1366.66         1370.66         693.84         13           3         86.10         426.30         408.27         398.29         443.31         L         1289.65         1271.62         1272.61         1273.61         645.32         12           4         88.04         541.32         523.30         513.32         558.34         D         1176.55         1155.54         1155.52         1160.52         588.77         11           5         87.06         655.35         637.34         627.36         672.38         N         1061.52         1043.51         1045.50         531.26         10           6         88.04         770.39         752.37         742.39         787.41         D         947.48         292.47         930.45         931.45         474.24         9           7         86.10         883.46         855.45         855.47         90.49         13.86         702.34         703.34         360.18         7           9         72.08         1039.55         1021.54         1015.65         N         662.35         644.34	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	1       198.15       216.15       198.14       188.16       233.17       144.10207@A       1366.66       1369.66       1370.66       693.84       13         3       88.10       426.30       408.27       398.29       443.31       L       1289.66       1370.66       645.32       122         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1158.54       1159.52       1160.52       588.77       11         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         9       72.08       109.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6         10       60.04       1126.59       1108.58 </td <td>1       198.15       216.15       199.14       188.16       233.17       144.10207@A       184.16       134.12         2       70.07       313.20       295.19       285.20       330.23       P       1366.66       1369.66       1370.66       693.84       13         3       88.10       426.30       408.27       398.29       443.31       L       1289.66       1370.66       645.32       122         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1159.52       1160.52       588.77       110         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1045.50       531.45       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       931.45       474.24       9         7       86.10       803.46       805.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         9       72.08       1009.55       1021.54       101.56       105.58       V       662.35       644.34</td> <td>Peptide</td> <td>Align Peptide D</td> <td>etails</td> <td></td>	1       198.15       216.15       199.14       188.16       233.17       144.10207@A       184.16       134.12         2       70.07       313.20       295.19       285.20       330.23       P       1366.66       1369.66       1370.66       693.84       13         3       88.10       426.30       408.27       398.29       443.31       L       1289.66       1370.66       645.32       122         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1159.52       1160.52       588.77       110         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1045.50       531.45       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       931.45       474.24       9         7       86.10       803.46       805.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         9       72.08       1009.55       1021.54       101.56       105.58       V       662.35       644.34	Peptide	Align Peptide D	etails											
1         188.15         216.15         198.14         188.16         233.17         144.10207@A             14           2         70.07         313.20         295.19         285.20         330.23         P         1386.69         1366.66         1370.66         693.84         13           3         86.10         426.30         408.27         398.29         443.31         L         1289.65         1271.62         1272.61         1273.61         645.32         12           4         88.04         541.32         523.30         513.32         558.34         D         1176.55         1158.54         1159.52         1160.52         588.77         11           5         87.06         655.35         637.34         627.36         672.38         N         1061.52         1043.51         1045.50         531.26         10           6         88.04         770.39         752.37         742.39         787.41         D         947.48         292.47         930.45         931.45         474.24         9           7         86.10         883.46         865.45         92.47         90.45         931.45         474.24         9 <td><math display="block"> \begin{array}{ c c c c c c c c c c c c c c c c c c c</math></td> <td>1       198.15       216.15       198.14       188.16       233.17       144.10207@A          14         2       70.07       313.20       295.19       285.20       330.23       P       1366.66       1370.66       693.84       13         3       88.10       446.30       408.27       398.29       443.31       L       1289.66       1272.61       1273.61       645.32       122         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1158.54       1159.52       158.57       104.50       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       547.25       282.14       5         10       60.04       1125.53&lt;</td> <td>1       198.15       216.15       198.14       188.16       233.17       144.10207@A          14         2       70.07       313.20       295.19       285.20       330.23       P       1366.66       1369.66       1370.66       693.84       13         3       88.10       426.30       408.27       398.29       443.31       L       1289.66       1370.66       645.32       122         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1158.54       1159.52       588.77       111         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1045.50       531.45       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       931.45       474.24       9         7       86.10       803.46       805.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.36</td> <td>#</td> <td>Immonium</td> <td>Ь</td> <td>Ь-Н2О</td> <td>а</td> <td>с</td> <td>Se</td> <td>a</td> <td>l v</td> <td>v-H2O</td> <td>z</td> <td>z'</td> <td>y (2+)</td> <td>#</td>	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	1       198.15       216.15       198.14       188.16       233.17       144.10207@A          14         2       70.07       313.20       295.19       285.20       330.23       P       1366.66       1370.66       693.84       13         3       88.10       446.30       408.27       398.29       443.31       L       1289.66       1272.61       1273.61       645.32       122         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1158.54       1159.52       158.57       104.50       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       547.25       282.14       5         10       60.04       1125.53<	1       198.15       216.15       198.14       188.16       233.17       144.10207@A          14         2       70.07       313.20       295.19       285.20       330.23       P       1366.66       1369.66       1370.66       693.84       13         3       88.10       426.30       408.27       398.29       443.31       L       1289.66       1370.66       645.32       122         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1158.54       1159.52       588.77       111         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1045.50       531.45       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       931.45       474.24       9         7       86.10       803.46       805.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.36	#	Immonium	Ь	Ь-Н2О	а	с	Se	a	l v	v-H2O	z	z'	y (2+)	#
2       70.07       313.20       295.19       285.20       330.23       P       1386.69       1368.66       1369.66       1370.66       693.84       13         3       86.10       426.30       408.27       398.29       443.31       L       1289.65       1271.62       1272.61       1273.61       645.32       12         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1159.52       1160.52       558.77       11         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       104.50       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	2       70.07       313.20       295.19       285.20       330.23       P       1386.69       1368.66       1369.66       1370.66       693.84       13         3       86.10       426.30       408.27       396.29       443.31       L       1289.65       11271.62       1272.61       1272.61       166.52       166.52       1285.20       160.52       166.52       166.55       588.77       1155       51155.51       1160.52       11045.50       531.26       100         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       444.24       9         7       86.10       883.46       665.45       855.47       900.49       11       632.47       816.43 <t< td=""><td>2       70.07       313.20       295.19       285.20       330.23       P       1386.69       1369.66       1370.66       693.84       13         3       86.10       426.30       408.27       398.29       443.31       L       1289.65       11271.62       1272.61       1272.61       1272.61       1272.61       1272.61       1273.61       645.32       12         4       88.04       551.32       558.34       D       1176.55       1158.57       1160.52       1043.51       1044.50       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       665.45       855.47       900.49       118.22       1044.50       1045.50       531.26       10         8       30.03       940.49       92.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1015.58       1056.58       V       662.35       644.34       645.32       31.67       &lt;</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>1</td><td></td></t<>	2       70.07       313.20       295.19       285.20       330.23       P       1386.69       1369.66       1370.66       693.84       13         3       86.10       426.30       408.27       398.29       443.31       L       1289.65       11271.62       1272.61       1272.61       1272.61       1272.61       1272.61       1273.61       645.32       12         4       88.04       551.32       558.34       D       1176.55       1158.57       1160.52       1043.51       1044.50       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       665.45       855.47       900.49       118.22       1044.50       1045.50       531.26       10         8       30.03       940.49       92.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1015.58       1056.58       V       662.35       644.34       645.32       31.67       <													1	
3         86.10         426.30         408.27         398.29         443.31         L         1289.65         1271.62         1272.61         1273.61         645.32         12           4         88.04         541.32         523.30         513.32         558.34         D         1176.55         1158.54         1159.52         1160.52         588.77         11           5         87.06         655.35         637.34         627.36         672.38         N         1061.52         1043.51         1044.50         1045.50         531.26         10           6         88.04         770.39         752.37         742.39         787.41         D         947.48         92.94         93.45         931.45         474.24         9           7         86.10         883.46         865.45         855.47         900.49         1         832.47         814.44         815.43         816.43         416.73         8           8         30.03         940.49         922.48         912.49         957.51         G         719.38         701.36         702.34         703.34         360.18         7           9         72.08         1039.55         1010.56         10         662.35 </td <td><math display="block"> \begin{array}{ c c c c c c c c c c c c c c c c c c c</math></td> <td>3       86.10       426.30       408.27       398.29       443.31       L       1289.65       1271.62       1272.61       1273.61       645.32       12         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1155.54       1155.55       505.31       160.52       588.77       11         5       87.06       655.35       667.33       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.32       120       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       1       832.47       816.43       816.43       116.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       703.34       360.18       7         9       72.08       1039.55       1101.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6</td> <td>3       86.10       426.30       408.27       398.29       443.31       L       1289.65       1271.62       1272.61       1273.61       645.32       12         4       88.04       541.32       523.30       513.32       558.34       D       1076.55       1158.54       1159.52       1160.52       588.77       11         5       87.06       655.35       667.33       627.36       672.38       N       106.52       103.51       1044.50       1045.50       531.32       120       105.52       1531.32       121       64.32       121.62       1272.61       1273.61       645.35       550.53       121.62       1272.61       1272.61       1272.61       1273.62       123.62       104.50       105.52       105.51       104.155       104.50       1045.50       103.15       104.14       815.43       816.43       416.73       8       8       30.03       940.49       927.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1101.56       1056.58       V       662.35       644.34       646.32       331.67       6         10       60.04       1255.63       1237</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>6.69 1368.</td> <td>66 1369.6</td> <td>6 1370.66</td> <td>693.84</td> <td></td>	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	3       86.10       426.30       408.27       398.29       443.31       L       1289.65       1271.62       1272.61       1273.61       645.32       12         4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1155.54       1155.55       505.31       160.52       588.77       11         5       87.06       655.35       667.33       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.32       120       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       1       832.47       816.43       816.43       116.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       703.34       360.18       7         9       72.08       1039.55       1101.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6	3       86.10       426.30       408.27       398.29       443.31       L       1289.65       1271.62       1272.61       1273.61       645.32       12         4       88.04       541.32       523.30       513.32       558.34       D       1076.55       1158.54       1159.52       1160.52       588.77       11         5       87.06       655.35       667.33       627.36       672.38       N       106.52       103.51       1044.50       1045.50       531.32       120       105.52       1531.32       121       64.32       121.62       1272.61       1273.61       645.35       550.53       121.62       1272.61       1272.61       1272.61       1273.62       123.62       104.50       105.52       105.51       104.155       104.50       1045.50       103.15       104.14       815.43       816.43       416.73       8       8       30.03       940.49       927.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1101.56       1056.58       V       662.35       644.34       646.32       331.67       6         10       60.04       1255.63       1237										6.69 1368.	66 1369.6	6 1370.66	693.84	
4         88.04         541.32         523.30         513.32         558.34         D         1176.55         1158.54         1159.52         1160.52         588.77         11           5         87.06         655.35         637.34         627.36         672.38         N         1061.52         1043.51         1044.50         1045.50         531.26         10           6         88.04         770.39         752.37         742.39         787.41         D         947.48         929.47         930.45         931.45         474.24         9           7         86.10         883.46         865.45         855.47         900.49         I         832.47         814.44         815.43         816.43         416.73         8           9         72.08         1039.55         1021.54         1011.56         1056.58         V         662.35         644.34         645.32         646.32         331.67         6           10         60.04         1126.59         1108.58         1098.59         1143.61         5         563.30         545.27         546.25         547.25         282.14         5           11         102.06         1255.63         1237.62         1227.63	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1158.54       1159.52       1160.52       588.77       11         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.46       447.42       9         7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       101.56       1056.58       V       663.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4	4       88.04       541.32       523.30       513.32       558.34       D       1176.55       1158.54       1159.52       1160.52       588.77       11         5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.26       10         6       88.04       770.39       752.37       742.39       737.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         9       72.08       1039.55       101.56       1056.58       V       662.35       644.34       645.23       263.67       231.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4								L						
5         87.06         655.35         637.34         627.36         672.38         N         1061.52         1043.51         1044.50         1045.50         531.26         10           6         88.04         770.39         752.37         742.39         787.41         D         947.48         929.47         930.45         931.45         474.24         9           7         86.10         883.46         865.45         855.47         900.49         I         832.47         814.44         815.43         816.43         416.73         8           8         30.03         940.49         922.48         912.49         957.51         G         719.38         701.36         702.34         703.34         360.18         7           9         72.08         1039.55         1021.54         1011.56         1056.58         V         662.35         644.34         645.32         331.67         6           10         60.04         1126.59         1108.58         1038.59         1143.61         5         563.30         545.27         546.25         547.25         282.14         5           11         102.06         1255.63         1237.62         1227.63         1272.66 <td< td=""><td><math display="block"> \begin{array}{ c c c c c c c c c c c c c c c c c c c</math></td><td>5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.26       10         6       68.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       92.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1015.6       1056.58       V       662.35       644.34       645.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       S       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4</td><td>5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.26       10         6       68.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       665.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       92.48       957.51       G       719.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1015.58       V       662.35       644.34       645.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       236.62       4         11       10.00       600       800&lt;</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.26       10         6       68.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       92.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1015.6       1056.58       V       662.35       644.34       645.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       S       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4	5       87.06       655.35       637.34       627.36       672.38       N       1061.52       1043.51       1044.50       1045.50       531.26       10         6       68.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       665.45       855.47       900.49       1       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       92.48       957.51       G       719.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1015.58       V       662.35       644.34       645.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       236.62       4         11       10.00       600       800<														
6         88.04         770.39         752.37         742.39         787.41         D         947.48         929.47         930.45         931.45         474.24         9           7         86.10         883.46         865.45         855.47         900.49         I         832.47         814.44         815.43         816.43         416.73         8           8         30.03         940.49         922.48         912.49         957.51         G         719.38         701.36         702.34         703.34         360.18         7           9         72.08         1039.55         1021.54         1011.56         1056.58         V         662.35         644.34         646.32         331.67         6           10         60.04         1126.59         1108.58         1098.59         1143.61         5         553.30         545.27         546.25         547.25         282.14         5           11         102.06         1255.63         1237.62         1227.63         1272.66         E         476.25         458.24         459.22         460.22         238.62         4	6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       S       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         9       200       400       600       800       1000       1200       1400       1600	6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       855.47       900.49       I       882.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1015.58       10056.58       V       666.35       646.32       331.67       6         10       60.04       1125.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         90       400       600       800       1000       1200       1400       1600         11:1       2X       2Y       200       400       600       800       100	6       88.04       770.39       752.37       742.39       787.41       D       947.48       929.47       930.45       931.45       474.24       9         7       86.10       883.46       865.45       885.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6         10       60.04       1126.59       1108.58       1099.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       459.22       460.22       238.62       4         9       200       400       600       800       1000       1200       1400       1600														
7         86.10         883.46         865.45         855.47         900.49         I         832.47         814.44         815.43         816.43         416.73         8           8         30.03         940.49         922.48         912.49         957.51         G         719.38         701.36         702.34         703.34         360.18         7           9         72.08         1039.55         1021.54         1011.56         1056.58         V         662.35         644.34         645.32         646.32         331.67         6           10         60.04         1126.59         1108.58         1098.59         1143.61         S         563.30         545.27         546.25         547.25         282.14         5           11         102.06         1255.63         1237.62         1227.63         1272.66         E         476.25         458.24         459.22         460.22         238.62         4	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       331.67       6         10       60.04       1126.59       1039.55       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         10       200       400       600       800       1000       1200       1400       1600	7       86.10       883.46       865.45       855.47       900.49       I       832.47       814.44       815.43       816.43       416.73       8         8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       331.67       6         10       60.04       1126.59       1039.55       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       102.06       1255.63       1237.62       1277.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       200       400       600       800       1000       1200       1400       1600         111       2X       2Y														
8         30.03         940.49         922.48         912.49         957.51         G         719.38         701.36         702.34         703.34         360.18         7           9         72.08         1039.55         1021.54         1011.56         1056.58         V         662.35         644.34         645.32         646.32         331.67         6           10         60.04         1126.59         1108.58         1098.59         1143.61         S         563.30         545.27         546.25         547.25         282.14         5           11         102.06         1255.63         1237.62         1227.63         1272.66         E         476.25         458.24         459.22         460.22         238.62         4	8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         1       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         1       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         1       102.0       400       600       800       1000       1200       1400       1600	8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       102.0       400       600       800       1000       1200       1400       1600         111       2X       2Y       200       400       600       800       1000       1200       1400       1600	8       30.03       940.49       922.48       912.49       957.51       G       719.38       701.36       702.34       703.34       360.18       7         9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         4       70 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>I</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>8</td>								I						8
9         72.08         1039.55         1021.54         1011.56         1056.58         V         662.35         644.34         645.32         646.32         331.67         6           10         60.04         1126.59         1108.58         1098.59         1143.61         5         563.30         545.27         546.25         547.25         282.14         5           11         102.06         1255.63         1237.62         1227.63         1272.66         E         476.25         458.24         459.22         460.22         238.62         4	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         ***********************************	9       72.08       1039.55       1021.54       1011.56       1056.58       V       662.35       644.34       645.32       646.32       331.67       6         10       60.04       1126.59       1108.58       1098.59       1143.61       5       563.30       545.27       546.25       547.25       282.14       5         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         ***********************************	8				912.49			G					360.18	7
11 102.06 1255.63 1237.62 1227.63 1272.66 E 476.25 458.24 459.22 460.22 238.62 4	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	11     102.06     1255.63     1237.62     1227.63     1272.66     E     476.25     458.24     459.22     460.22     238.62     4	11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4         11       102.06       1255.63       1237.62       1227.63       1272.66       E       476.25       458.24       459.22       460.22       238.62       4	9	72.08	1039.55	1021.54	1011.56	1056	5.58	٧	662	2.35 644.3	34 645.3	2 646.32	331.67	6
	E	Find the second	Find the second	10	60.04	1126.59	1108.58	1098.59	1143	3.61	S	563	3.30 545.2	27 546.2	5 547.25	282.14	5
		Find the second	Find the second	11	102.06	1055.40	1227.62	1227.63	1273	266	F	47/	25 450 2	450.2	2 460.22	238.62	4
		200 400 600 800 1000 1200 1400 1600	200 400 600 800 1000 1200 1400 1600		102.08	1235.63	1237.62	1227.03				4/0			100.22		<u> </u>
Info Survey Alignment Error Map	0.1				1:1 2X 2Y Survey Alignmen	200						<u> </u>			y13		
Info Survey Alignment Error Map	0.1				1:1 2X 2Y Survey Alignmen	200						<u> </u>			y13		

## 4) Running Quantification

Select the PEAKS Search result file, and click the PEAKS Quantification toolbar icon Q or selecting "Quantification" from the "Tools" menu. The quantification parameters window will open. Enter the parameters as shown below and click "OK".

📐 Quantification		Once completed, the
Tools	Quantification	protein
. Data Refine . De novo	Basic Options Mass Error Tolerance: 0.1 Da  Upper Bound of Precursor Charge: 4	quantification result will be displayed in the same PEAKS
. PEAKS Search	Label Options	Protein ID result
. SPIDER Search	Labelling occurs at the M5/M5 level eg. ITRAQ     Labelling occurs at the M5/evel eg. ICAT	window that you selected earlier.
. PTM Finder	Clabel-Free	
✓ Quantification	Sample         Reporter Ion (Da)         Labelling Efficiency (%)           51         114.112         1.0           52         117.115         1.0	The results are listed as a "Ratio of 117.115:114.112" and as "Standard
	Add Label Delete Label	Deviation of
		117.115:114.112". They are highlighted in the
		red box below. For example the relative protein ratio for the
	OK Cancel Help	top ranked protein is 4.73 with a standard

derivation of 0.38.

DB Search         Image: Cold and	Accessio		ID I	Aass I	PEAKS(Scor	. Ratio 11	7.115:114.112	SD 117.115:114	h112 [	Display	Coverag	Query mat.	Markeo	Des
Sequence Browser         Sequence Comparison           NCBI BLAST search of         000222054L ECC01           Link to retrieve entries containing this sequence from NCBI Entree:         Accession/ID           Pop2220564L ECC01         Beta-galactosidase OS=Escherichia coli (strain K12) GN=lac2 PE=1 SV=2           Pop2220564L ECC01         Beta-galactosidase OS=Escherichia coli (strain K12) GN=lac2 PE=1 SV=2           Pop220585         Sequence         PEAKS(Sc, RSD         M/Z         Z         117.115.114.112         Mr(Caic)         Delta(Ma         Error(p, File         RT         Scan           Peptides         Spectrum 1         A(2)PLDNDIGVSEATR         9.0         0.0         801.388         2         4.99         1600.8176         0.0552         36.9196/TIRAQ5an         0.042           Spectrum 1         A(2)PLDNDIGVSEATR         9.0         0.0         400.756         2         4.99         1600.8176         0.0522         35.0785/TIRAQ5an         0.025           Spectrum 1         T(2)PLONDUTQUR         92.26         0.0         786.887         2         4.59         1571.7815         0.022         13.9797/TIRAQ5an         0.782           Spectrum 5         V(2)DEDQEPFRAVEX[3]         50.99         0.71         815.43         2         4.55         1628.8652												_		
NCBI BLAST search of \$0072218GAL_ECOL         Link to retrieve entries containing this sequence from NCBI Entrez:         Accession/ID         Description         Poptides List:         ID       Sequence       PEAKS(Sc, RSD       M/Z       117.115:114.112       Mr(Calc)       Detacle Accession/ID         Peptides List:         ID       Sequence       PEAKS(Sc, RSD       M/Z       117.115:114.112       Mr(Calc)       Deta(Ma, Error(p, File       RT       Scan         Peptides <tb colspan="2">Peptides         Septrum 1       A[2]/PhONDIGVSEATR       9.0       0.0       80       80.0       4.94       1600.8176       0.022       3.5.0785 ITRAQSam       0.022       3.5.0785 ITRAQSam       0.022       3.5.0785 ITRAQSam       0.022       3.5.0785 ITRAQSam       0.22       4.59       15.5       0.022       3.5.0785 ITRAQSam       0.22</tb>	🚊 🕕 PU	JU722[BGAL_EC	OLI I	116482.8	96.5	9	4.	38	0.34 L		4.3	à	5	Beta-
NCBI BLAST search of @00722 BGAL_ECOL         Link to retrieve entries containing this sequence from NCBI Entrez:         Accession/ID       Description         P0722 BGAL_ECOL       Beta-galactosidase OS=Escherichia coli (strain K12) GN=lac2 PE=1 SV=2         Peptides List:       ID       Sequence         Peptides       Spectrum 1       A[2]PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 ITRAQSam       0.042         Spectrum 1       A[2]PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 ITRAQSam       0.042         Spectrum 5       D[2]VENPGVTQUNR       99.2       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 ITRAQSam       0.042         Spectrum 5       D[2]VENPGVTQUNR       99.2       0.03       467.727       2       4.69       1571.7815       0.022       13.9797 ITRAQSam       0.761.8         Spectrum 8       V[2]DEDQPFPAVPK[3]       50.99       0.71       815.43       2       4.55       1628.8652       0.0198       12.1407 ITRAQSam       0.761.3         Matched peptides shown in blue, SPIDER matches shown in red         2														
NCBI BLAST search of @00222IBGAL_ECOL         Link to retrieve entries containing this sequence from NCBI Entrez:         Accession/ID       Description         P00722]BGAL_ECOLI       Beta-galactosidase 05=Escherichia coli (strain K12) GN=lacZ PE=1 SV=2         Poptides List:       D         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 4       TQ1/FISR       71.38       0.0       440.756       2       4.77       879.52997       0.025       36.196 (TRAQSam       0.042         Spectrum 5       D[2]WENPGVTQUNR       92.26       0.0       786.887       2       4.59       1571.7815       0.022       13.9797 (TRAQSam       0.276         Spectrum 7       M[2]SGIPR       49.21       0.33       427.727       2       5.43       853.46027       0.0038       24.3872 (TRAQSam       0.76 13         Matched peptides shown in blue, SPIDER matches shown in red       2       1       1.51628.8652       0.0198       12.1407 (TRAQSam														
NCBI BLAST search of @00222IBGAL_ECOL         Link to retrieve entries containing this sequence from NCBI Entrez:         Accession/ID       Description         P00722]BGAL_ECOLI       Beta-galactosidase 05=Escherichia coli (strain K12) GN=lacZ PE=1 SV=2         Poptides List:       D         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 4       TQ1/FISR       71.38       0.0       440.756       2       4.77       879.52997       0.025       36.196 (TRAQSam       0.042         Spectrum 5       D[2]WENPGVTQUNR       92.26       0.0       786.887       2       4.59       1571.7815       0.022       13.9797 (TRAQSam       0.276         Spectrum 7       M[2]SGIPR       49.21       0.33       427.727       2       5.43       853.46027       0.0038       24.3872 (TRAQSam       0.76 13         Matched peptides shown in blue, SPIDER matches shown in red       2       1       1.51628.8652       0.0198       12.1407 (TRAQSam														
NCBI BLAST search of 600222IBGAL_ECOL         Link to retrieve entries containing this sequence from NCBI Entrez:         Accession/ID       Description         Po0722/BGAL_ECOLI       Beta-galactosidase 05-Escherichia coli (strain K12) GN-lacZ PE-1 SV-2         Peptides List:       ID         Sequence       PEAKS(Sc         Peptides       117.115:114.112         Mr(Calc)       Delta(Ma         Error(p       File         RT       Scan         Opeptides       117.115:114.112         Mr(Calc)       Delta(Ma         Error(p       File         RT       Scan         Spectrum 4       12/PLDNDIGVSEATR         9.0       0.0       801.388       2         4.34       1600.8176       0.0562       35.0785 ITRAQ5am       0.025         Spectrum 4       12/PLSTBR       71.38       0.0       440.756       2       4.77       879.52997       0.0252       35.0785 ITRAQ5am       0.256         Spectrum 5       D[2]WENPGVTQLNR       92.26       0.0       786.887       2       4.59       1571.7815       0.022       13.9797 ITRAQ5am       0.258         Spectrum 7       M[2]SGIFR       49.21       0.33       4														
NCBI BLAST search of @00222IBGAL_ECOL         Link to retrieve entries containing this sequence from NCBI Entrez:         Accession/ID       Description         P00722]BGAL_ECOLI       Beta-galactosidase 05=Escherichia coli (strain K12) GN=lacZ PE=1 SV=2         Poptides List:       D         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 4       TQ1/FISR       71.38       0.0       440.756       2       4.77       879.52997       0.025       36.196 (TRAQSam       0.042         Spectrum 5       D[2]WENPGVTQUNR       92.26       0.0       786.887       2       4.59       1571.7815       0.022       13.9797 (TRAQSam       0.276         Spectrum 7       M[2]SGIPR       49.21       0.33       427.727       2       5.43       853.46027       0.0038       24.3872 (TRAQSam       0.76 13         Matched peptides shown in blue, SPIDER matches shown in red       2       1       1.51628.8652       0.0198       12.1407 (TRAQSam														
NCBI BLAST search of @00222IBGAL_ECOL         Link to retrieve entries containing this sequence from NCBI Entrez:         Accession/ID       Description         P00722]BGAL_ECOLI       Beta-galactosidase 05=Escherichia coli (strain K12) GN=lacZ PE=1 SV=2         Poptides List:       D         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 1       A(2)PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 (TRAQSam       0.042         Spectrum 4       TQ1/FISR       71.38       0.0       440.756       2       4.77       879.52997       0.025       36.196 (TRAQSam       0.042         Spectrum 5       D[2]WENPGVTQUNR       92.26       0.0       786.887       2       4.59       1571.7815       0.022       13.9797 (TRAQSam       0.276         Spectrum 7       M[2]SGIPR       49.21       0.33       427.727       2       5.43       853.46027       0.0038       24.3872 (TRAQSam       0.76 13         Matched peptides shown in blue, SPIDER matches shown in red       2       1       1.51628.8652       0.0198       12.1407 (TRAQSam	Sequence	a Promor C-		1										
Link to retrieve entries containing this sequence from NCBI Entrez:           Accession/ID         Description           P00722/BGAL_ECOLI         Beta-galactosidase OS=Escherichia coli (strain K12) GN=lacZ PE=1 SV=2           Peptides List:           D         Sequence         PEAKS(Sc, RSD         M/Z         Z         117.115:114.112         Mr(Calc)         Delta(Ma, Error(p, File         RT         Scan           Peptides          Spectrum 1 A[2]PLDNDIGVSEATR         99.0         0.0         801.388         2         4.94         1600.8176         0.0562         35.0785 ITRAQSam         0.042           Spectrum 5         T12JFISR         71.38         0.0         440.756         2         4.77         879.52997         0.0325         36.9196 ITRAQSam         0.428           Spectrum 5         V[2]VENPGVTQLNR         92.26         0.0         786.887         2         4.69         1571.7815         0.0228         24.3872 ITRAQSam         0.428           Spectrum 5         V[2]VENPGVTQLNR         92.26         0.0         786.887         2         4.55         1628.8652         0.0198         12.1407 ITRAQSam         0.428           Spectrum 5         V[2]VENPGVTQLNR         92.26         0.0         71	Sequenc	Le browser Se	quence Comparison											
Link to retrieve entries containing this sequence from NCBI Entrez:           Accession/ID         Description           P07722/BGAL_ECOL1         Beta-galactosidase OS=Escherichia coli (strain K12) GN=lac2 PE=1 SV=2           Peptides List:           ID         Sequence         PEAKS(Sc, RSD         M/Z         Z         117.115:114.112         Mr(Calc)         Delta(Ma         Error(p, File         RT         Scan           Peptides          Spectrum 1         A(2)PLDNDIOVSEATR         99.0         0.0         801.388         2         4.94         1600.8176         0.0562         35.0785 ITRAQSam         0.042           Spectrum 1         A(2)PLDNDIOVSEATR         99.0         0.0         801.388         2         4.94         1600.8176         0.0562         35.0785 ITRAQSam         0.042           Spectrum 5         A(2)PLDNEOVSEATR         99.0         71.38         0.02         1.39797 ITRAQSam         0.428           Spectrum 5         VEXPNSVTQLINR         92.26         0.0         766.87         2         4.69         1571.7815         0.022         13.9797 ITRAQSam         0.428           Spectrum 7         M(2)SGER         49.21         0.33         427.727         2         5.43         853.	NCBI B	LAST search of	PODZ221BGAL_ECO											
Accession/ID         Description           P00722/BGAL_ECOLI         Beta-galactosidase OS=Escherichia coli (strain K12) GN=lac2 PE=1 SV=2           Peptides List:         ID         Sequence         PEAKS(Sc, RSD         M/Z         Z         117.115:114.112         Mr(Calc)         Delta(Ma         Error(p, File         RT         Scan           Peptides         • Spectrum 1         A[2]PLDNDIGVSEATR         99.0         0.0         801.388         2         4.94         1600.8176         0.0562         35.0785 iTRAQSam         0.042           • Spectrum 1         T[2]FISEN         71.38         0.0         440.756         2         4.77         879.52997         0.0325         36.9196 iTRAQSam         0.428           • Spectrum 5         0[2]FISEN         49.21         0.33         427.727         2         5.43         853.46027         0.0208         24.3872 iTRAQSam         0.428           • Spectrum 8         V[2]DEDQPFPAVPK[3]         50.99         0.71         815.43         2         4.55         1628.8652         0.0198         12.1407 iTRAQSam         0.761.3           Matched peptides shown in blue, SPIDER matches shown in red         2         1         4.55         1628.8652         0.0198         12.1407 iTRAQSam         0.761.3 <td></td> <td></td> <td></td> <td>_</td> <td>otrez:</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>				_	otrez:									
Poor22[BGAL_ECOL1         Beta-galactosidase OS=Escherichia coli (strain K12) GN=lac2 PE=1 SV=2           Peptides List:         Image: Constraint of the straint of			concarning this sequ	ioneo n'om webi e			1							
Peptides List:         Image: Decision of the system o														
D         Sequence         PEAKS(Sc         RSD         M/Z         Z         117.115:114.112         Mr(Calc)         Delta(Ma         Error(p         File         RT         Scan           Peptides <ul></ul>	P00722	2 BGAL_ECOLI					Bel	ta-galactosidase OS=	=Escherichia o	oli (strain K12)	GN=lacZ PE=	=1 SV=2		
D         Sequence         PEAKS(Sc         R5D         M/Z         Z         117.115:114.112         Mr(Calc)         Delta(Ma         Error(p         File         RT         Scan           Peptides          Spectrum 1         A[2]PLDNDIGVSEATR         99.0         0.0         801.388         2         4.94         1600.8176         0.0562         35.07851TRAQSam         0.042           Spectrum 5         D[2]WENPGVQLNR         92.26         0.0         786.887         2         4.69         1571.7815         0.022         13.97971TRAQSam         0.428           Spectrum 7         M[2]SGIFR         49.21         0.33         427.727         2         5.43         853.46027         0.0208         24.38721TRAQSam         0.7613           Spectrum 8         V[2]DEDQPFPAVPK[3]         50.99         0.71         815.43         2         4.55         1628.8652         0.0198         12.14071TRAQSam         0.7613		-												
Peptides         4.9         1600.8176         0.0562         35.0785 iTRAQ5am         0.042           • Spectrum 1         T[2]PLDNDIGVSEATR         99.0         0.0         801.388         2         4.94         1600.8176         0.0562         35.0785 iTRAQ5am         0.042           • Spectrum 5         D[2]WENPGVTQLNR         92.26         0.0         786.887         2         4.69         1571.7815         0.0221         3.9797 iTRAQ5am         0.256           • Spectrum 7         M[2]SGIFR         49.21         0.33         427.727         2         5.43         853.46027         0.0208         24.3872 iTRAQ5am         0.5911           • Spectrum 8         V[2]DEDQPFFAVFK[3]         50.99         0.71         815.43         2         4.55         1628.8652         0.0198         12.1407 iTRAQ5am         0.7613	Peptid	les List:												
• Spectrum 1       A[2]PLDNDIGVSEATR       99.0       0.0       801.388       2       4.94       1600.8176       0.0562       35.0785 iTRAQSam       0.042         • Spectrum 4       T[2]LFISR       71.38       0.0       440.756       2       4.77       879.52997       0.0325       36.9166 iTRAQSam       0.256         • Spectrum 5       D[2]WENPGVTQLNR       92.26       0.0       786.887       2       4.69       1571.7815       0.022       13.9797 iTRAQSam       0.256         • Spectrum 7       M[2]SGIFR       49.21       0.33       427.727       2       5.43       853.46027       0.020       24.3872 iTRAQSam       0.428         • Spectrum 8       v[2]DEDQPFFAVPK[3]       50.99       0.71       815.43       2       4.55       1628.8652       0.0198       12.1407 iTRAQSam       0.76 13	ID		Sequence	PEAKS(Sc	RSD M	1/Z	z	117.115:114.112	Mr(Calc)	Delta(Ma	Error(p	File	RT S	ican
• Spectrum 4       T[2]LFISR       71.38       0.0       440.756       2       4.77       679.52997       0.0325       36.9196/TRAQSam       0.256         • Spectrum 5       D[2]WENPGVTQLNR       92.26       0.0       786.887       2       4.69       1571.7815       0.022       13.9797/TRAQSam       0.428         • Spectrum 7       M[2]SGIFR       49.21       0.33       427.727       2       5.43       853.46027       0.0208       24.3872/TRAQSam       0.428         • Spectrum 8       V[2]DEDQPFPAWPK[3]       50.99       0.71       815.43       2       4.55       1628.8652       0.0198       12.1407/TRAQSam       0.7613	🛅 Pej	ptides												
• Spectrum 5       D[2]WENPGVTQLNR       92.26       0.0       786.887       2       4.69       1571.7815       0.022       13.9797 iTRAQsam       0.428         • Spectrum 7       M[2]SGIFR       49.21       0.33       427.727       2       5.43       853.46027       0.0208       24.3872 iTRAQsam       0.428         • Spectrum 8       V[2]DEDQPFPAVPK[3]       50.99       0.71       815.43       2       4.55       1628.8652       0.0198       12.1407 iTRAQsam       0.76 13         Matched peptides shown in blue, SPIDER matches shown in red         2       2       1       MTMITDSLAV VLQRRDWENP GVTQLNPLAA HPPFASWRNS EEARTDRPSQ         51       QLRSLNGEWR FAWFFAPEAV PESWLECDLP EADTVVVPSN WQMHGYDAPI       51       QLRSLNGEWR FAWFFAPEAV PESWLECDLP EADTVVVPSN WQMHGYDAPI				99.0	0.0	801.388				0.0562	35.0785	iTRAQSam		
Spectrum 7 M[2]SGIFR 49.21 0.33 427.727 2 5.43 853.46027 0.0208 24.3872.ITRAQSam 0.5911 Spectrum 8 V[2]DEDQPFPAVPK[3] 50.99 0.71 815.43 2 4.55 1628.8652 0.0198 12.1407.ITRAQSam 0.7613 Matched peptides shown in blue, SPIDER matches shown in red 2 Matched peptides shown in blue, SPIDER matches shown in red 2 1 MITMITDSLAV VLQRRDWENP GVTQLNRLAA HPPFASWRNS EEARTDRPSQ 51 QLRSLNGEWR FAWFFAPEAV PESWLECDLP EADTVVVPSN WQMHGYDAP1						440.756		4.77	879.52997	0.0325				
Spectrum 8 V[2]DEDQPFPAVPK[3] 50.99 0.71 815.43 2 4.55 1628.8652 0.0198 12.1407 ITRAQSam 0.76 13 Matched peptides shown in blue, SPIDER matches shown in red 2 1 MIMITDSLAV VLORRDWENP GVTOLNELAA HPPFASWENS EEARTDRPSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLP EADTVVVPSN WQMHGYDAPI														
Matched peptides shown in blue, SPIDER matches shown in red 2 1 MTMITDSLAV VLQRRDWENP GVTQLNFLAA HPPFASWRNS EEARTDRPSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLP EADTVVVPSN WQMHGYDAPI														
2 1 MIMIIDSLAV VLQRRDWENP GVIGLNFLAA HPPFASWRNS EEARIDRFSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLF EADIVVVPSN WOMHGYDAFI		Spectrum 8	/[2]DEDQPFPAVPK[3	3] 50.99	0.71	815.43	2	4.55	1628.8652	0.0198	12.1407	iTRAQSam	0.7613	
2 1 MIMIIDSLAV VLQRRDWENP GVIGLNFLAA HPPFASWRNS EEARIDRFSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLF EADIVVVPSN WOMHGYDAFI														
2 1 MIMIIDSLAV VLQRRDWENP GVIGLNFLAA HPPFASWRNS EEARIDRFSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLF EADIVVVPSN WOMHGYDAFI														
2 1 MIMIIDSLAV VLQRRDWENP GVIGLNFLAA HPPFASWRNS EEARIDRFSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLF EADIVVVPSN WOMHGYDAFI														
2 1 MIMIIDSLAV VLQRRDWENP GVIGLNFLAA HPPFASWRNS EEARIDRFSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLF EADIVVVPSN WOMHGYDAFI														
1 MTMITDSLAV VLQRRDWENP GVTQLNRLAA HPPFASWRNS EEARTDRPSQ 51 QLRSLNGEWR FAWFPAPEAV PESWLECDLP EADTVVVPSN WQMHGYDAPI		Matched p		, SPIDER matche	s shown in re	)d								
51 QLRSINGEWR FAWFPAPEAV PESWLECDLP EADTVVVPSN WOMHGYDAPI			2											
	1	MTMITD:	SLAV VLORRDM	ENP GVTOLNR	LAA HPPF	ASWRNS	EEARTDRPSC	2						
			_					-						
	51	QLRSLN	GEWR FAWFPAP	EAV PESWLEC	DLP EADT	VVVPSN	WQMHGYDAPI	[						

## 15.4 SILAC Walkthrough

Stable isotope labeling with amino acids in cell culture (SILAC) is a method to metabolically label proteins for relative quantitative comparison. One cell population is fed amino acids of normal isotopic composition; the other cell population is fed amino acids labeled with heavier isotopes. The heavy amino acids are incorporated into newly synthesized proteins, eventually completely replacing the cells' proteins, such that labeling efficiency is near 100%. The cell populations are then mixed together and digested for MS analysis to determine differential protein abundances.

#### 1) Creating a project

Click on the "Create new project" icon **C** or select "New project" from the "File" menu. The following window will appear:

Steps	Project Properties	;
1. Project Properties	Project Name:	SILAC Sample
2	Project Location:	C:\Peaks Studio 5.0\.\derbyServer\serverDB Browse
	Project Folder:	Yeaks Studio 5.0\.\derbyServer\serverDB\SILAC Sample
	Notes/Description:	
	Type and organizatio	n of project:
	Type and organizatio	n of project:
	<ul> <li>Basic Project</li> </ul>	n of project: illed samples for comparison (each sample can be fractionated)
	<ul> <li>Basic Project</li> </ul>	

Give your project a name, such as SILAC Sample. Then click "Next". The following window will appear. Give your sample a name such as SILAC 1.

Click the "Add a file for this sample" button and select the file "C:/PEAKS 5/Data/SILACSample.mzxml". Click "OK" to add this to the list of selected files.

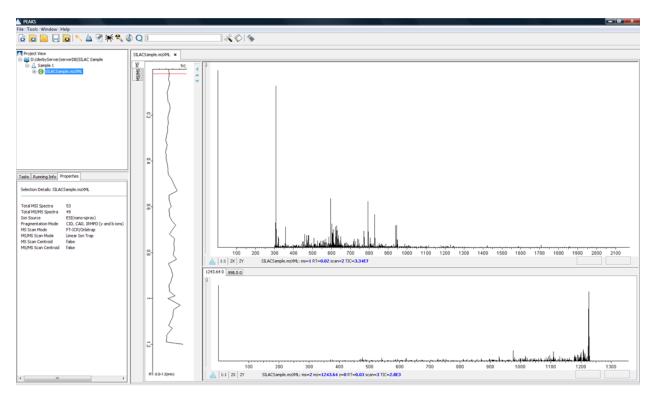
🖄 New Project		×
Steps	Sample Properties	
1. Project Properties	Give this sample a name: SILAC 1	~
2. Sample Properties	Select Files:	
3	Name Size Date Modified Type	[
	Add a file for this sample Remove file from list Clear list	
	Sample Notes/Description:	
	Add another sample Remove current sample	
	<< Back Next >> Cancel Help	

For cases where you want to add another sample to the project, select "Add another sample" and repeat these last two steps. In our case we are not going to add any more samples, so we can just click "Next".

📐 New Project	
<ul> <li>New Project</li> <li>Steps</li> <li>Project Properties</li> <li>Sample Properties</li> <li>Instrument Details</li> <li></li> </ul>	Instrument Details         Instruments(s) used to acquire the data:         Thermo Scientific         LTQ FT Ultra Hybrid FT-F°         LTQ FT Ultra Hybrid FT-T°         Default Parent Mass Tolerance:         0.01 Da         Default Parent Mass Tolerance:         0.5 Da         MS Data Centroided:         Yes         MS/MS Data Centroided:         Yes         Add a new instrument
	< Back Next >> Cancel Help

The following ection will tell PEAKS which ype of mass pectrometer was used to generate he data. This ample was lerived from a Thermo LTQ Drbitrap. Click he box beside LTQ FT Ultra Hybrid FT-Trap" o select this nstrument.

Upon clicking "Next" a sample project will be created. Your "Main Processing Screen" should look something like this:



## 2) Running Protein Identification

1) In the *"Project View Frame"*, select SILACSample.mzxml Click the data

refinement tool 🌂	🖄 Data Refine		
select "Data Refine"	Tools	Data Refine	
from the "Tools" menu. Enter the parameters as shown below and click "OK".	✓ Data Refine . De novo . PEAKS Search . SPIDER Search . PTM Finder	Merge Options Merge scans of the same peptide: Retention time window: (for raw files only) m/z tolerance: Charge Options Correct precursor charges:	yes ono min. Da ♥
	, Quantification	Minimum charge:     1     Max       Filter Options     Filter MS/MS scans:        Precursor mass between	ximum charge:     3       O yes     no       and     Da       and     min       suggest 0.65
		Preprocess Options Preprocess MS/MS scans:	_ no, already done
			OK Cancel Help

### 3) Running Protein Identification

Click the PEAKS Search toolbar icon or select "PEAKS Search" from the Tools menu.

The Protein Identification Parameters dialogue window will appear. Enter the following parameters:

	Database Search		Save Parameter	SILAC	•
	Mass Options			General Opti	ons
. Data Refine	Parent Mass Error Tolerance:	0.1 Da 🗸	Precursor Mass Search Type	: Preproce	ss this data 'on the fly'
. De novo	Fragment Mass Error Tolerance:	0.8 Da	Monoisotopic O Ave	rage Max Missed	Cleavages: 3 🚔
10000000000000000	Enzyme Options				
PEAKS Search	Enzyme:	Trypsin		•	New Enzyme
cororo casado		[			
. SPIDER Search	Digest Rule:	RK	{P}		Delete Enzyme
. PTM Finder	Find peptides that satisfythe	s above rule at both en	ds	[	Advance
. Quantification					
Contra cocorri	PTM Options			Fixed Modification	1
	Name Mono mass	Residue site	Add Fixed =>	<ul> <li>Exect Modification</li> <li>Carbamidomethyla</li> </ul>	tion
	Myristoylation 210.1984	[KC], [G]@N	Remove	+ corporation(city)	
	N-acyl diglyceri 788.7258	[C]			
	N-isopropylcar 99.0684	[C]			
	N-Succinimidyl 127.0633	[K], [X]@N		Undahla Madifiantian	
	Oxidation M 15.9949	[M]	Add Variable =>	Variable Modification	
	Oxidation HW 15.9949	[HW]		• K6	
	Palmitovlation 238.2297	[CSTK]	<= Remove	Oxidation M	
	Phosphopante 340.0858	[5]	-		
	Show unimod	New PTM		Max variable PTM per pe	eptide: 3 🜩
	Database Options				
	Select database				2445
	Select database	elect Database: Sampl	leDB	all species	
	Paste fasta sequences				
		ive.	w Database Edit Databa		
	Advanced Options				
		inique that requires sor	me sequence tags to help in the	e search	
	PEAKS uses a hybrid search tech		me sequence tags to help in the	e search	
			me sequence tags to help in the	e search	
	PEAKS uses a hybrid search tech	, don't run it again		e search Thermo_1	•
	PEAKS uses a hybrid search ted I have already run de novo Run de novo using different	, don't run it again t parameters than the a	bove		•]
	PEAKS uses a hybrid search ted I have already run de novo Run de novo using different Run de novo using the same	, don't run it again t parameters than the a	bove		•
	PEAKS uses a hybrid search ted I have already run de novo Run de novo using different	, don't run it again t parameters than the a	bove		•]
	PEAKS uses a hybrid search ted I have already run de novo Run de novo using different Run de novo using the same	, don't run it again t parameters than the a	bove		•
	PEAKS uses a hybrid search ted I have already run de novo Run de novo using different Run de novo using the same	, don't run it again t parameters than the a	bove		•]
	PEAKS uses a hybrid search ted I have already run de novo Run de novo using different Run de novo using the same Validation - decoy search	, don't run it again t parameters than the a	ibove (default)		•

Click "OK". This will launch PEAKS Protein ID and when completed click on the "Protein View" button. The results should appear similar, to those shown below:

View	Accession	ID	Mass	Display	PEAKS(Score	Coverage(%)		Marked	Description	
\$	DB Search									~
ů,	sp P21333 FLNA_HUMAN	1	280736.56		98.89	2.53	6		Filamin-A OS=Homo sapiens GN=F	
Der	sp Q9NZM1 MYOF_HUMAN	2	234706.47		59.35	1.21	2		Myoferlin OS=Homo sapiens GN=F	
View		3	23034.43		34.43	5.24	1		Cobalt-precorrin-8X methylmutase	
		6	66017.65		33.33	4.97	3		Keratin, type II cytoskeletal 1 OS=	
beptide		308	149313.25		25.73	1.04	1		DNA-directed RNA polymerase sub	🔳
		309	78610.53		22.24	1.53	1		Glycyl-tRNA synthetase beta subu	
View		245	191587.25		20.59	0.54	1		Clathrin heavy chain 1 OS=Bos tau	
	😟 🔘 sp A5A6M6 K2C1_PANTR	5	65489.13		20.1	1.1	2		Keratin, type II cytoskeletal 1 OS=	
otein		9	278192.7		19.43	0.42	2		Filamin-B OS=Homo sapiens GN=F	. –
<u>م</u>		13	16317.283		15.61	4.26	1		Protein E6 OS=Human papillomavir	
	🗌 🔿 logozecipulas, aperu	240	27/0/ 500		10.17	272			*	

Note that the top protein result is Human Filamin A, with a score of 98.89%.

### 4) Running Quantification

Select the PEAKS Protein ID result file, and click the PEAKS Quantification toolbar icon Q or selecting "Quantification" from the "Tools" menu. The quantification parameters window will open. Enter the parameters as shown below and click "OK".

Quantification				
Tools	Quantification			
	Basic Options			
, Data Refine	Mass Error Tolerance:	0.1	Da 💉 Upper Bound	of Precursor Charge: 4 📚
. De novo	Retention Time Range:	1.0	min. 💌	
PEAKS Search	Label Options			
SPIDER Search	O Labelling occurs at th	he MS/MS level eg. iTRA	Q	
PTM Finder	Labelling occurs at the constant of the con	he MS level eg. ICAT		
Quantification	Sample	Added Mass	Residues	Labelling Efficiency (
	Heavy Light		6.0 K 0.0K	1.0
		<u> </u>	<u> </u>	
		Add Label	Delete Label	

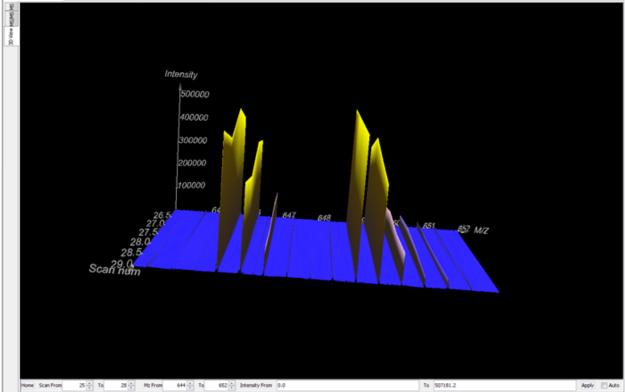
Once completed, the protein quantification result will be displayed in the same PEAKS Protein ID result window that you selected earlier. The results are listed as a "Ratio Heavy: Light" and "Standard Deviation Heavy: Light". They are highlighted in the red box below. For example, the highest ranked protein, Human Filamin-A has a ratio of 1.2 and a standard deviation of 0.1

		±									
Accession	ID	Mass	(	Display	PEAKS(Score %)	Coverage(%)	Ratio Heavy:Light	SD Heavy:Light	Query matched	Marked	Description
DB Search											
- @ P21333 FLNA	_HU	61	280736.56		] 98.95	2.53	1.18	0.1		6 📃	Filamin-A (Alpha-filamin
P04264 K2C1_	JHU	62	66017.65		34.7	4.97			-	3 📃	Keratin, type II cytoske
Q9Y283 INV5_	HU	64	117826.305		31.33	0.94	19.83	. 0		1 📃	Inversin (Inversion of e
Q8N6N7 ACBD	07_ł	66	9790.278				1.09	0		1 📰	Acyl-CoA-binding domai
Q96EU6 CF15		67	29823.105		13.72		10.49	0		1 📃	UPF0399 protein C6orf
P13497 BMP1	HU	68	111248.77		11.87	0.81	0.49	0		1 📰	Bone morphogenetic pr
Q7Z460 CLAP	1_H	69	169449.78		10.32	0.65				1 📰	CLIP-associating protei
Q8N5Z0 AADA		70	47351.652		9.85					1	Kynurenine/alpha-amin
Q96EP1 CHFR		71	73386.586		8.33					1	E3 ubiquitin-protein liga.
O7E2601ELNIP											Cilemia D (CINID) (Deba

There are four peptide features are identified and calculated for quantification of Human Filamin-A.

Accession/ID						Description							
21333 FLNA_HUMAN						Filamin-A (Alpha-	filamin) (Filamin-1)	(Endothelial acti	n-binding prote	in) (Actin-binding pro	tein 280) (AB	P-280) (Nonm	uscle filamin) - H.
eptides List:													
ID	Sequence	PEAKS(Score %)	M/Z	Z	Heavy:Light	Mr(Calc)	Delta(Mass)	Error(ppm)	RSD	File	RT	Scan	Quality
le Peptides													
Spectrum 12	YGGQPVPNFPSK[3]	99.0	648.84	2	1.05	1295.6604	-0.0051	3.957		0.92 SILACSample		0.2827	0.
Spectrum 13	DVDIIDHHDNTYTVK[3]	67.65	895.94	2	1.22	1789.8579	-0.0076	4.2285		0.87 SILACSample		0.2928	0.
Spectrum 19	TGVELGK[3]PTHFTVNAK[3]	99.0	855.99	2	1.28	1709.9502	-0.0153	8.9234		- SILACSample		0.3938	0.
Spectrum 27	DVDIIDHHDNTYTVK	46.55	595.62	3	1.17	1783.8376	-5.0E-4	0.2737		0.93 SILACSample		0.8871	0.
Spectrum 35	VANPSGNLTETYVQDR	99.0	882.44	2		1762.8486	-0.0168	9.5559		0.25 SILACSample		1.0787	0.
Spectrum 44	VEPGLGADNSVVR	99.0	1312.6899	1		1311.6782	-0.0044	3.3503		0.54 SILACSample		1.22102	0

PEAKS provides the 3D view of each peptide feature for visual validation. For example, spectrum 12 is among the feature of YGGQPVPNFPSK. Input the scan range of  $25 \sim 28$ , and m/z range of  $644 \sim 652$ . Click the button of "Apply". The 3D view of the feature is displayed.



# 15.3 Label Free Quantification (Available in future)

Label Free quantification relies on the changes in analyte signals directly reflecting their concentrations in one sample relative to another. This technology employs overall spectral intensity normalization by interpreting signals of molecules that do not change concentration from sample to sample. By comparing two or more spectra, PEAKS can determine the constant intensity ratio between the unchanging analytes forms the basis for identifying the non-changing concentrations, making spiking unnecessary.



# 16. References

#### De novo

Bin Ma, Kaizhong Zhang, Christopher Hendrie, Chengzhi Liang, Ming Li, Amanda Doherty-Kirby, Gilles Lajoie. PEAKS: Powerful Software for Peptide *De novo* Sequencing by MS/MS. Rapid Communications in Mass Spectrometry, 17(20):2337-2342. 2003.

### **SPIDER**

Y. Han, B. Ma, and K. Zhang. SPIDER: Software for Protein Identification from Sequence Tags Containing *De novo* Sequencing Error. Journal of Bioinformatics and Computational Biology 3(3):697-716. 2005.

### Quantification

Yang, W., Chen, W., Rogers, I., Ma, B., Bendall, S., Lajoie, G., Smith, D., PEAKS Q: Software for MSbased quantification of stable isotope labeled peptides. Bioinformatics Solutions Inc., Genome BC Proteomics Centre, University of Western Ontario. ASMS 2006 poster WP531.

Chapter

# 18. Appendix

# 18.1 Terminology and Abbreviations Glossary

**a-ions**: an N-terminal fragment holding at least one charge. This is a fragment of the peptide derived from b-ions. The a-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, subtract the mass of carbon monoxide.

**b-ions**: an N-terminal fragment holding at least one charge. This is a prefix fragment of the peptide. The b-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues.

BSI (Bioinformatics Solutions Inc.): The makers of PEAKS and other fine bioinformatics software.

**c-ions**: an N-terminal fragment holding at least one charge. This is a prefix fragment of the peptide. The c-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, plus the mass of ammonia.

**Deconvolution**: rearrangement of the spectrum to show each monoisotopic peak as if it were singly charged. Thus, to reposition them on the scale, PEAKS multiplies the m/z of ion's that were doubly charged by two minus the mass of 1 H. Note that the deconvolved scale PEAKS shows is 'at +1.'

**Fixed modification**: selecting a post-translational modification as a fixed modification tells PEAKS that this modification is applied to all occurrences of the residue(s) on which the PTM can occur.

**Enzyme**: Biomolecules that catalyze chemical reactions, including the digestion of proteins.

ESI (Electrospray Ionization): A method for ionizing a sample into the mass spectrometer.

*m/z*: mass to charge ratio.

**MALDI** (Matrix-Assisted Laser Desorption/Ionization): A method for ionizing a sample into the mass spectrometer. This has a characteristic effect of producing singly charged ions.

**Mass accuracy**: this refers to the accuracy of data obtained from a given mass spectrometer. On a spectrum, this is reflected by how close the peaks are to the actual masses of the ions they represent.

**PTM** (**Post-Translational Modification**): A newly translated protein may differ from its final form as a result of processing by various enzymes in the cellular environment. This change is referred to as a post-translational modification. Since PTMs change the mass of residues, it must be accounted for when sequencing peptides by mass spectrometry.

**Built-in PTM**: PEAKS comes equipped with a library of possible post-translational modifications. These can be incorporated into a *de novo* analysis at the click of a button.

**Customized PTM**: If the post-translational modification you are looking for is not in the PEAKS PTM set, you may create our own entry, or modify an existing one. This will appear as a customized PTM in the set.

**PTM library**: A listing of all possible (built-in and custom entered) post-translational modifications that PEAKS can use as a part of its analysis.

**Residue**: as used in this manual, a residue refers to what remains of an amino acid once it has become part of a peptide, or peptide fragment. In this manual, residues are referred to by their original amino acid names.

**Resolution**: refers to the resolving power of an instrument. On a spectrum, this is reflected by how close together two peaks can be and still be resolved.

**Variable modification**: selecting a post-translational modification as a variable modification tells PEAKS that this modification may or may not be applied to the residue(s) on which the PTM can occur.

**x-ions**: a C terminal fragment holding at least one charge. The x-ion's mass will be the sum of the masses of the C terminal group, plus the intervening neutral amino acid residues, plus the mass of carbon monoxide.

**y-ions**: a C terminal fragment holding at least one charge. The y-ion's mass will be the sum of the masses of the C terminal group, plus the intervening neutral amino acid residues, plus the mass of 2 H.

**z-ions**: a C terminal fragment holding at least one charge. The z-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, after subtracting the mass of ammonia.

# **18.2** Toolbars

### Main Window Toolbar

New File: This allows you to open a new data file created by the mass spectrometer in its native format, or a PEAKS data file (in ANZ format) that also contains peptide analysis data. Other accepted file formats include PKL, DTA, MGF or ANZ.

Close File: Selecting this icon will remove the presently selected data file from its project.

New Project: Clicking the New Project button will allow users to create a brand new project, offering organization to any study.

Open Recent Project: The quickest method to recalling an existing project. Users are instantly directed to a selection of recently created, modified or viewed PEAKS projects.

Close Project: When a project is complete or a user temporarily no longer is working on a particular project, users can click this icon to remove it from their Project View.

Save As: The project method of PEAKS allows for all results to be automatically constantly saved. However, if a user wishes to save a particular application under a new heading this is the most convenient and appropriate way to do so. Just click the icon and enter the new title in the field provided. The file will be saved in the ANZ format. Press this after selecting a data file in the Peptide Data Frame.

Print: Whether a user desires to print their ms spectrum views to complement a publication or print matrices, the print feature offers a straight forward connection to any printer configured to the user's computer.

Export: Easily export the spectrum view, ion table, or a picture (bmp, gif, or jpg format) with ions, masses, PEAKS and peptides marked.

Exit: To exit from the PEAKS software safely, select this icon or press on the keyboard the 'Control' key simultaneously with the letter 'Q'.

Solution Data Refine: Merge scans of the same peptide, remove noise spectra, preprocess within each MS/MS spectrum and recover peptide charge state. The data refinement options dialogue will allow us to choose and to set parameters for each of these refinement tools.

*De novo*: Perform auto *de novo* for a selected data file, spectrum or project. Press this after selecting one or more data files (or spectra) in the Peptide Data Frame. An auto *de novo* options dialogue will allow users to set parameters before beginning.

PEAKS Search: Perform protein identification on a selected data project. Press this after selecting one or more data files (or spectra) in the Peptide Data Frame. A protein identification options dialogue will allow users to set parameters.

 $\bigstar$  SPIDER Search: Peptide homologue search tool.

PTM Finder: A PTM finder search can be performed on any PEAKS Protein ID and allows the user to be able to identify more PTMs in less time.

inChorus Search: inChorus, a meta protein identification tool, can be used to compare and validate results, calling upon such search engines as Mascot, OMSSA, PEAKS, Sequest and X!Tandem, as well as SPIDER. Click this icon to set a task to have inChorus perform a user's analysis easily.

Quantification: Where users require abundance ratio results, PEAKS Q provides insight. Click here to analyze results from ICAT, iTRAQ, Label Free, SILAC, N-terminal and User Defined Labeling techniques.

Configuration: Set up enzymes, post translational modifications, databases, instruments and parameters.

Preferences: Organize general properties such as directories, search engines and ion editing capabilities.

Mass Calculator: The Mass Calculator is a simple tool to help us determine the molecular weight of a peptide. Clicking this icon will make the mass calculator appear.

#### **Project View**

The project view allows optimal organization and greater control when managing multiple files at once. As different projects may be active at once, it is important to understand the different categories and levels presented by PEAKS.

Tree Root
 Project Node
 Filter Result
 Sample Node
 File Node
 Combine Results
 Compare Results

Search Engines: Within the Project View, these icons are used to represent which method/search engine a particular file was interpreted by.



## Main Processing Window Toolbar

Market Profile Mode

- I Peak Mode
- <sup>1:1</sup> Return to original size

<sup>2X</sup> Zoom X-axis

<sup>2</sup>Y Zoom Y-axis

# **18.3 Mass Calculator**

Click the Mass Calculator icon on the toolbar  $\bigcirc$ Or

choose Mass Calculator from the Tools menu.

The following window will appear:

🗞 PEAKS 📃 🗖 🔀
File Help
Input Sequence or Mass (Da): 🔿 b-ion 🔿 y-ion 💿 non-specific
Peptide Mass:       Charge:       M/Z Mass:         0.00       <-
Sequences: Mass (Da):
Advanced Calculate! Clear

In order to use the mass calculator input the peptide sequence or enter the mass in Daltons. Indicate if the sequence contains b- or -y ions or if your search is non-specific.

For example select "b-ion", input the sequence ACDR and click the "Calculate" button. You should see the following:

Input Sequence (	or Mass (Da):	💿 b-ion	🔘 y-ion	🔘 non-specific
ACDR				
Peptide Mass:	irge:	M/2	Z Mass:	
446.182	<- 1		-> 44	6.182

You can change the charge and use the arrow on the right to calculate the precursor mass or use the arrow on the left to calculate the peptide mass.

Or for another example, change the margin of error to 1 Da, input a mass of 146 Da and click the "Calculate" button. In the sequences box you should see the following predicted sequences:

Sequences:	Mass (Da):
GS	144.0535
SG	144.0535

Use the "Clear" button to start again.

#### **Advanced Options**

Clicking on the "Advanced" button will display the following:

Advanced Options									
A C D E F G H I K L									
M N P Q R S T V W Y									
Water Proton PTM Back Clear									
Modification	Mass	Residues	Include						
Acetylation (N-te	42.010567	[ADCEQGILM	~						
Acetylation (K)	42.010567	[K]@Anywhere							
Amidation	-0.984016	[ARNDCEQG							
Applied Biosyste	442.225	[C]@Anywhere							
Applied Biosyste	450.2752	[C]@Anywhere							
Applied Biosyste	227.12698	[C]@Anywhere	×.						
unimod De	lete PTM	New PTM	Edit PTM						

You will now be able to add various modifications to your amino acid sequence. For example, enter A, M, then select "Oxidation on M" from the PTM list and click the "PTM" button. Then click the "Calculate button. You should see the following:

Input Sequence	or Mass (Da):	💿 b-ion	🔘 y-ion	🔘 non-specific
AM <ox m=""></ox>				
Peptide Mass:	Char	rge:	M/2	Z Mass:
219.08	<- 1		-> 21	9.08

To view additional PTMs from the unimod list, select the "unimod" box. You can delete a PTM using the "Delete PTM" button. You can also edit or create a new PTM using the "Edit PTM" or "New PTM" buttons, respectively.

You can also use the advanced options menu to add a water or proton to your sequence. The "Back" button will remove the last amino acid or modification that you have added to your sequence or click on the "Clear" button to start again.

Chapter

# **19** About Bioinformatics Solutions Inc.

BSI provides advanced software tools for analysis of biological data.

Bioinformatics Solutions Inc. develops advanced algorithms based on innovative ideas and research, providing solutions to fundamental bioinformatics problems. This small, adaptable group is committed to serving the needs of pharmaceutical, biotechnological and academic scientists and to the progression of drug discovery research. The company, founded in 2000 in Waterloo, Canada, comprises a select group of talented, award-winning developers, scientists and sales people.

At BSI, groundbreaking research and customer focus go hand in hand on our journey towards excellent software solutions. We value an intellectual space that fosters learning and an understanding of current scientific knowledge. With an understanding of theory, we can focus our talents on providing solutions to difficult, otherwise unsolved problems that have resulted in research bottlenecks. At BSI, we are not satisfied with a solution that goes only partway to solving these problems; our solutions must offer something more than existing software.

The BSI team recognizes that real people will use our software tools. As such, we hold in principle that it is not enough to develop solely on theory; we must develop with customer needs in mind. We believe the only solution is one that incorporates quality and timely results, a satisfying product experience, customer support and two-way communication. So then, we value market research, development flexibility and company-wide collaboration, evolving our offerings to match the market/user's needs.

Efficient and concentrated research, development, customer focus and market analysis have produced: PEAKS software for protein and peptide identification from tandem mass spectrometry data, RAPTOR and PROSPECT Pro software for threading based 3D protein structure prediction and PatternHunter software for all types of homology search sequence comparison.

Chapter 119

# 20 PEAKS Software License

This is the same agreement presented on installation. It is provided here for reference only.

If we are evaluating a time limited trial version of PEAKS and we wish to update the software to the full version, we must purchase PEAKS and obtain a full version registration key.

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9. Maintenance and Support. BSI will provide technical support for a period of thirty (30) days from the date the Software is shipped to Licensee. Further maintenance and support is available to subscribers of BSI's Maintenance plan at BSI's then current rates. Technical support is available by phone, fax and email between the hours of 9 am and 5 pm, Eastern Time, excluding statutory holidays.

10. Governing Law. This Agreement shall be governed by and construed in accordance with the laws in force in the Province of Ontario and the laws of Canada applicable therein, without giving effect to conflict of law provisions and without giving effect to United Nations Convention on contracts for the International Sale of Goods.



Brou

For technical support issues not found in this manual, please contact either your Sales Representative or any of our support service resources:

email: support@bioinfor.com tel: (519) 885-8288 fax: (519) 885-9075 online: www.bioinfor.com/peaks