

User Manual

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# Chapter

#### 1 WELCOME TO



#### 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 search 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 ultimately find peptides with interesting sequence variations or modifications that would prevent them otherwise being identified. Our multi-engine protein identification search tool, inChorus allows users to utilize multiple search engines (PEAKS, X!Tandem, OMSSA, Sequest and Mascot) to expand sequence coverage and increase confidence. A key feature unique to PEAKS is SPIDER, a homology search tool for *de novo* sequencing. SPIDER is able to reconstruct the correct sequence using the *de novo* sequence and a homologous peptide.

#### 1.2 New Features

Throughout this manual, new and existing features will be discussed in considerable detail. Below is a short list of features implemented in PEAKS 5.2:

- Increased de novo and database search accuracy for ETD data
- CID/HCD, CID/PQD support in iTRAQ quantification with Thermo instruments
- Automatic precursor mass and charge correction for high resolution data
- MCP complaint result exporting
- New GUI for result comparison
- Added replicate analysis for label free quantification
- Improved workflows targeting different application needs such as Identification and Quantification
- Overall improvement of software capacity, stability and speed

- Improved spectrum display
- mzML/mzXML3.0/mzData/pepXML file format support
- Standardized PTM display
- Better support for Thermo LTQ RAW file format
- Adjusted SPIDER scoring function
- Reduced the memory overhead for SPIDER, improving its capacity and speed
- Search parameters of other search engines used in inChorus can now be saved and used at a later time
- PTM support in manual de novo
- Options to display assigned as well as unassigned de novo peptides in PEAKS Protein ID results
- Added FASTA database validation, important for custom databases

#### 1.3 GUIDELINES FOR USING THIS MANUAL

This manual is intended to assist in the use of PEAKS 5.2. It outlines functionalities, provides instruction on how to customize PEAKS to a specific application, provides a task based reference, and offers troubleshooting recommendations. It is highly recommend that users begin by going through the included Walkthrough (Chapter 3) and use the sample data provided to quickly become familiar with most commonly used features of PEAKS.

#### 1.4 Scope

It is assumed that PEAKS users are familiar and comfortable with using computers and their respective operating systems. Given this, 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 one of the numerous computer help books for such information. Similarly, PEAKS users are expected to be familiar with mass spectrometry, standard operating practices, data acquisition and analysis.

#### 1.5 SERVICE AND SUPPORT

In addition to reading this manual, it is recommended that users take the time to view the accompanying training videos that explain the main features of PEAKS visually and in detail.

http://www.bioinfor.com/products/peaks/support/tutorials.php

Please send technical questions to support@bioinfor.com.

We strongly encourage users to provide BSI with any suggestions or comments, as BSI is consistently improving and updating PEAKS to meet the future needs of the scientific community.

http://www.bioinfor.com/corporate/contactform.php

# Chapter 2

#### 2 GETTING STARTED WITH PEAKS<sup>®</sup> 5.2

This section of the manual will guide users through the installation and configuration of PEAKS 5.2.

#### 2.1 PACKAGE CONTENTS

The PEAKS 5.2 package contains:

- This manual
- PEAKS 5.2 software

#### 2.2 System Requirements

PEAKS 5.2 will run on most platforms with the following minimum requirements:

- Processor: Equivalent or superior processing power to an Intel<sup>TM</sup> Pentium 4 Processor 1.6GHz (we recommend an Intel<sup>TM</sup> Core2 Duo processor/AMD Phenom<sup>TM</sup> processor)
- Memory: 1 GB memory (we recommend 2GB memory)
- Operating System: PEAKS runs and has been tested on Windows XP, Vista and 7.

#### 2.2.1 ADJUSTING THE AMOUNT OF MEMORY UTILIZED BY PEAKS

The PEAKS directory (e.g., C:\PeaksStudio5.2) contains a file called Memory Utility.exe. Click to open this file (it also can be accessed from the Windows Start Menu) and the following window will open:

The default of 1,024 MB tells the Java Virtual Memory (which runs PEAKS) to execute with 1,024 MB of memory. You can increase the Java VM to determine the highest optimal value for your computer. Keep in mind that trial and error may be needed as Java will not start if you set the value to be too large for your system. Please do not hesitate to contact BSI if you have any questions about your own system.



#### 2.3 INSTALLATION FOR WINDOWS USERS

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

- 1) Close all programs that are currently running.
- 2) Insert the PEAKS 5.2 disc into the CD-ROM drive. If you are installing PEAKS via the download link, move ahead to step 4, after downloading and running the file.
- The installation window should automatically appear after the CD-ROM is inserted. If it does not, find the CD-ROM drive and open it to access the disc. Double-click on PEAKS\_Studio\_Installation.exe.
- 4) A menu screen will appear. Select the top item "PEAKS Installer." The installation utility will launch the installer. When the "PEAKS 5.2" installation dialogue appears, click the "Next" button.



- 5) Basic system requirements will be presented. Please note that while the minimum requirement is 1 GB of RAM, the preferred configuration is 2 GB of RAM. Click "Next".
- Read the license agreement. If you agree, select "I accept the terms of the License Agreement" and click "Next".
- 7) Choose the folder/directory in which you would like to install PEAKS. The default location is simply "C:\PeaksStudio5.2". To change this location press the "Choose..." button to browse your system directory and make your selection, or type a folder name into the textbox. Avoid installing PEAKS in any directory that contains a white space, for example the "Program Files" directory as some features may not function correctly in such situation. Please make sure that the user account has full access permissions (read/write/execute) on the selected directory. Click "Next".
- 8) Choose where you would like to place icons for PEAKS 5.2. The default location for the icons is in the Programs section of your Start Menu. A common user preference is on the desktop. Click "Next".
- 9) The Pre-Installation Summary will allow you to review the selections you have made. You can click "Previous" if you would like to make any changes or click "Next" if you are satisfied with all your specifications.
- 10) PEAKS 5.2 will now install on your system. You may cancel the process at any time by simply pressing the "Cancel" button located in the lower left corner.

11) When the installation is complete, click "Done". The PEAKS 5.2 menu screen should still be open. You may view tutorial movies and other materials from here. To access this menu at a future date, simply insert the disc in your CD-ROM drive.

#### 2.4 REGISTERING PEAKS

To complete the registration, the user should have the following information ready.

- A valid email address The license email will be sent to this address.
- A registration key The key usually is a 20-character-long alphanumeric string and it is case-sensitive. The registration key is not needed when registering for a 30-day evaluation license.

### Also during the registration, it is recommended to input ONLY English characters as other character encodings may corrupt the license file.

The first time PEAKS is run, the license wizard will appear automatically. There are three options. The first two options should be used to start the registration process. The third option should be used when a user reinstalls/re-registers the software on the same computer and still has the license file (license.lcs) retrieved from the license email.

#### 2.4.1 REGISTRATION INSTRUCTIONS (WITH INTERNET

#### CONNECTION)

If the computer has internet connection, please follow the following instructions.

- 1) Select "Request license file (has Internet connection)" and click "Next".
  - a. The following window will appear:



- b. If you have purchased PEAKS and have a registration key, select "Registration Key". Enter your registration key as well as your name and email address and click "Next".
- OR
- c. If you are trying a demo of PEAKS and do not have a registration key, select "Request a 30 day evaluation license (No registration key required)". Enter your name, email address, as well as your institution. Click "Next".

K License Wizard	8
Your Name	
Email Address	
Email (confirm)	
Important: You will receiv	ve your license file via email
Registration Key	
🔘 Request a 30 days e	valuation license (No registration key required)
Institution	
<< Back	Next >> Cancel Help

2) If the license file is requested successfully, the following window will appear:

🔊 License Wizard	83
Registration failed	
The license key is not valid. Please contact BSI (support@bioinfor.com) to solve issue.	the
<< Back Finish Cancel Help	

- 3) An automated BSI service will generate the license file (license.lcs) and send an email (from support@bioinfor.com) to the email address provided from the License Wizard. You can either save the attached license file you receive to a local directory or copy the content between '===>' and '<===' in the email. Click "Next" and go to step 4.</p>
- 4) If the following error message appears, it means the input registration key is not correct. It is usually caused by a typo in the registration key field in step 2. The registration key is case-sensitive and it is recommended to copy (ctrl-C) / paste (ctrl-V) the registration key whenever possible.
- 5) If the registration failed with other error messages, please follow the on screen instructions or refer to section 2.4.2 Registration Instructions (Without Internet Connection).

🔊 License Wizard

Paste the license

- 6) The following window will appear:
- 7) Select "Paste the license" and click on the "Paste from Clipboard" button to paste the copied license information between '===>' and '<===' from the license email or select "Import the license file (the email attachment)" and browse to locate the license file (license.lcs). Click "Next".
- uUzalfDNzmaWbvG+tG1Y+J1L2W660zUv1eFrytQyyN4yLua9EMfvnOQTF+U
  </ 
  III 
  Import the license file (the email attachment)

  Cancel Help

Paste from Clipboard

8) The following window will open:

- 9) Click "Finish" if you receive a message that the license has been imported.
- 10) Restart PEAKS to begin using the software.

### 2.4.2 REGISTRATION INSTRUCTIONS (WITHOUT INTERNET CONNECTION)

If the computer does not have internet connection, or it is behind a proxy/firewall, or failed using the first option, please follow these instructions.

1) Select "Request license file (without Internet connection)" and click "Next". The following screen will describe the registration process:



- a. Obtaining a license.request file (using the onscreen guide)
- b. Uploading the request file to a web address via a computer with internet access
- c. Retrieving the license file (license.lcs) via email and importing it to activate the software.
- d. Click Next.
- 2) The following window will open: If you have purchased PEAKS and have a registration key, select "Registration Key". Enter your registration key as well as your name and email address and click "Next".

OR

If you are trying a demo of PEAKS and do not have a registration key, select "Request a 30 day evaluation license (Ne registration key required)" Enter your name amail address

(No registration key required)". Enter your name, email address, as well as your institution. Click "Next".

- 3) The following window will appear:
  - a. Select the "Save Request File" button to save license.request to your computer (PC1). Click "Next".

🛃 License Wizard		83
Your Name		
Email Address		
Email (confirm)		
Important: You will recei	ve your license file via email	
Registration Key		
💿 Request a 30 days e	valuation license (No registration key required)	
Institution		
<< Back	Next >> Cancel Help	

N License Wizard	X			
Click the button below to save the request file to a USB key or a floppy drive.				
Save Request File				
Copy the request file (license.request) to another computer (PC2) which has Internet connections.				
The next step will be performed on PC2.				
< <back next="">&gt; Cancel Help</back>				

- 4) Transfer the license.request file from your computer (PC1) onto a computer with an Internet connection (PC2) using a USB key or a removable storage device. On PC2, go to http://www.bioinfor.com/lcs20/index.jsp
- 5) On this website, select "I have the license request file. I want to register the software" and click "Next".
- 6) On the following window click the "Browse" button to select the license.request file on your USB key, then type in the visual verification code and click "Next". The web page may display:
  - a. Operation completed successfully. Go to step 7.

- b. Invalid product key. It means the input registration key is not correct. It is usually caused by a typo in the registration key field in step 2. The registration key is case-sensitive and it is recommended to copy (ctrl-C) / paste (ctrl-V) the registration key whenever possible. The user will have to go through the registration wizard again to generate a new license.request file with the correct registration key.
- c. Invalid verification code. Sometimes the characters in the image are not very clear therefore the visual verification failed. In this case, the user can click the Back button and start from step 5. The visual verification code is case-sensitive.
- d. Unknown error occurred. If this message is displayed, please contact support@bioinfor.com for additional assistance.
- After the license email is received on PC2, save the attachment, license.lcs, as is and transfer the file to your computer with PEAKS (PC1). If you do not receive the license.lcs file in your inbox, please check your junk mail folder.
- 8) In the license wizard on your computer (PC1), click the "Browse" button to select the license.lcs file and click "Next".
- 9) Click "Finish" you should receive a message that the license has been imported successfully.

N License Wizard	
License import completed	
The license file has been imported. The licens software is launched.	e will be verified the next time the
<< Back Finish	Cancel Help

10) Restart PEAKS to begin using the software.

#### 2.4.3 RE-REGISTRATION INSTRUCTIONS

Re-registering PEAKS may be necessary if you purchased additional software module or renewed SPS. BSI will modify the license information accordingly on the server side. You will need to obtain a new license file in order to make the changes effective. Select "About PEAKS" from the Help menu. The "About BSI PEAKS Studio" dialogue box will appear:

Click the "License Wizard" button to continue. Then follow the instructions previously listed above for reregistering PEAKS.

About BSI PEAKS Studio       PEAKS Studio 5.2         Copyright & 2000-2010 Bioinformatics Solutions Inc. All rights reserved.         Beavis is the author of the X/Tandem program. BSI is grateful to Dr. Beavis is the author of the X/Tandem program. BSI is grateful to Dr. Is grateful to Solutions Inc. (BSI) acknowledges that Romaid distributes X/Tandem in accordance with the following Artistics         Items for all X! software, binaries and documentation. BSI is motion of grateful to Dr. Is grateful to Dr. Is grateful to Dr. Is grateful to Dr. Is grateful to Solutions Inc. All rights reserved.         Leense for all X! software, binaries and documentation. BSI is not responsible for the performance of X/Tandem, and makes no warranty or grantante for it.         Leense to to grateful to Dr. Never Expires         Register enall leense start/ expire         Septier Expires         Start Baform       Never Expires         PEAKS De novo       Never Expires         PEAKS De Novo Rever Expires       PEAKS Q         Vew end user kense agreement       Warring. This compare, or eny will be prosecuted to the maximum extent possible under the lew.         Warring this compare, or eny will be prosecuted to the maximum extent possible under the lew.       Tech support										
FEXS Stude 5.2         Coynight © 2000-2010 Bioinformatics Solutions Inc. (BSI) a chronuledges that Ronald Events is the author of the X!Tandem program. BSI is grateful to Dr. Beavis is the author of the X!Tandem program. BSI is grateful to Dr. Beavis for allowing us to share X!Tandem with the following Attistic License for all X! Software, binaries and documentation. BSI is not users. BSI is createful to Dr. Beavis for allowing us to share X!Tandem switch coursents. BSI is createful to Dr. Beavis for allowing us to share X!Tandem with the following Attistic License for all X! Software, binaries and documentation. BSI is not users. BSI is createful to Dr. Beavis for allowing attistic traponsible for the performance of X!Tandes, and makes no warranty or or grantee for it.         Wernse to       PEAKS User's Name         License to       PEAKS Name         Register enall       email@address.com         Nodale       License status         PEAKS Maform       Never Expires         SPS sorie       No. No. Never Expires         SPEAKS Plaform       Never Expires         PEAKS Plaform       Never Expires         SPEAKS Plafor<	About BSI PEAKS Stud	lio				x				
License to         PEAKS User's Name           License key         PS####################################		PEAKS Studio 5.2 Copyright © 2000-2010 Bioin Bioinformatics So Beavis is the auth Beavis for allowi distributes X!Tand License for all X! responsible for th or quarantee for it	formatics Solutions Inc. () or of the XITande ng us to share em in accordanc software, binari e performance of )	All rights reserved. BSI) acknowl m program. BSI X!Tandem wi e with the es and documen X!Tandem, and	edges that Ronald is grateful to Dr. th our users. BSI following Artistic tation. BSI is not makes no warranty	•				
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#### 2.5 SET UP PEAKS PREFERENCES

Before running your data, you must set up search engine preferences. For an explanation please refer to section <u>16.1.3 Search Engine Preferences</u>.

It is possible to analyze your data with PEAKS without changing any of the preferences but the analysis will use the default settings of PEAKS. For more information on changing these default settings refer to section <u>16.1 PEAKS</u> <u>Environment Preferences</u>.

#### 2.6 SET UP PEAKS CONFIGURATION

Before running your data, you must configure the databases. For detailed instructions on the configuration process please refer to section <u>16.2.3 Database Configuration</u>.

For more information on changing these default settings refer to section 16.2 PEAKS Configuration.

### Chapter 3

#### 3 QUICK WALKTHROUGH

This section of the manual will walk you through the basic workflow of PEAKS 5.2. After completing this section you will see how easy it is to load, view a data file, perform data refinement, de novo sequencing, and protein identification via database searches.

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

#### 3.1 CREATE A SAMPLE DATABASE

Before beginning with the walkthrough, you need to set up a database. We have provided you with a sample FASTA database called "SampleDB.fasta" in your PEAKS program folder (C:\PeaksStudio5.2\Data).

Click on the configuration toolbar icon or select "Configuration" from the "Tools" menu. Select "Database" from the left hand side of the window. Under "Database Details" select UniProtKB/SwissProt from the dropdown list, and then call this database Sample DB. Locate the database using the browse button (C:/PEAKSStudio5.2/Data/SampleDB.fasta).

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 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 or select "New Project" from the "File" menu. The following window will appear:



roject Name	New Project 1		
roject Location	C:\PEAKS_Studio_5.2\.\der	byServer\serverDB	Browse
Data Files		Sample Details	
Test Data		Replicate	Sample Name
Add data fil	es	1 4	Sample 1
		Instruments	

Enter a name for your project. In this case, we can call it User Data Project. By default the first sample will be named "Sample 1". Click the "Add data files" button to add a data file for Sample 1. Select the "OrbiSample.mzxml" file from your PEAKS program folder (for example "C:\PEAKSStudio5.2\Data") and click "Open". Leave the instrument vendor as "All Instruments" and select "FT-trap" from the "Instrument Type" drop-down list. Click "OK".

As this project is created, we can see the project load in the "Project View" panel, which is located in the upper left hand corner of the screen. The icon to the left of the sample will change from outlined to a solid fill when the sample is ready to be analyzed.

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 the data file when choosing data to be analyzed, rather than just the project. As this is just a single sample project, there is no confusion, however, if multiple samples are loaded, clicking on the project will process them all. Selecting a single data file will ensure only the desired file is processed.

PEAKS reads and tracks information about the experiment and data for use in the current analysis and for future reference. Click on the "Properties" tab in the bottom left hand corner to view these features:

By double clicking on the loaded data file, we can 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 Chromatogram" (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 spectra that were generated from the tandem scan. For more information on the functions and tools found in these windows, refer to section 4.7 Orienting Yourself.

#### Tasks Running Info Properties Selection Details: C:/PeaksStudio5.2/./derbyServer/server Project Name User Data Project Total Samples 1 Instrument Info: Fractions: OrbiSample.mzXML Ton Source ESI(nano-spray) CID, CAD, IRMPD (y and b ions) Fragmentation Mode FT-ICR/Orbitrap MS Scan Mode MS/MS Scan Mode Linear Ion Trap >



#### 3.3 PERFORM DATA REFINEMENT

1) With the OrbiSample.mzXML file selected, click the

Data Refine toolbar icon <sup>5</sup> or select "Data Refine" from the "Tools" menu.

- 2) Enter the following settings:
  - Correct Precursor Mass
  - Correct Precursor Charge States (min 1, max 4)
  - Filter Scans: quality value greater than 0.65)
  - Data Preprocess: yes

For more details on setting up the parameters for data refinement refer to section <u>5.2 Data Refinement Parameters</u>.



Here a quality filter is used to remove spectra with a quality value lower than 0.65. As all of the spectra in this data set are of good quality, no data will be removed using this data filter. After executing data refinement, there will be new information listed in the "Properties" file.

Selection Details: OrbiSample.mzXI	ЧL	^
Total MSI Spectra	7	
Total MS/MS Spectra	9	
Ion Source	ESI(nano-spray)	
Fragmentation Mode	CID, CAD, IRMPD (y and b ions)	
MS Scan Mode	FT-ICR/Orbitrap	
MS/MS Scan Mode	Linear Ion Trap	
MS Scan Centroid	false	
MS/MS Scan Centroid	false	
Default data refine id	1	
Group MS/MS Scan (after refine)	9	
Charge Options	[1 - 4]	
Filter Quality	>0.65	
Process	true	
Default	true	

- Save as...

Set PTM...

Remove Switch type

Help

View Enzyme
 New Enzyme...

OK Cancel

Renove

we Al Switch Type

OK Cancel

#### 3.4 RUN DE NOVO SEQUENCING

1) Click the de novo sequencing toolbar icon and or select "de novo" from the "Tools" menu.

- 2) Enter these settings:
  - Error Tolerance:
  - Parent Ion to 20ppm
  - Fragment ion to 0.8 Da.
  - Enzyme: Trypsin

• PTM: To enter Carboxymethyl as a fixed PTM click the "Set PTM..." button and a new window will appear. Select Carboxymethyl from the list on the left and click the arrow on the right that corresponds with "Selected Fixed PTM". Then click OK.

- Max allowed variable PTM: 3
- Note that there is no need to preprocess this data set "on the fly" as we have already preprocessed the data during the data refinement stage.

You can save the parameters that you used for future

reference by clicking "Save As". For more information on setting up de novo parameters refer to section <u>6.1</u> <u>Setting up Auto de novo Sequencing Parameters</u>. Click "OK" to commence analysis.

De No

Tools

Data Ref

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SDIDED S

PTM Find

AI PTM

Replicate Anal

De Novo

Enzyme

Trypsin

F Carboon

General Option

rum allowed variable PTM per peptide 👘 3 ≑

Report up to 5 🚖 candidates per spectrum

ess this data on-the-fly (peak centroiding

Error Tolerance

Parent ion: 20

The PEAKS auto de novo algorithm derives sequence candidates for each of the nine spectra in our example data file. Once the de novo sequencing is completed the results file will appear in the "Project View Panel":

Double click on the de novo file and the results will appear in the "Main Processing Window":

Take a look at spectra ID 6. Notice that the (+58.01) refers to the modification which in this case is Carboxymethyl.

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 on the "Peptide Candidates Frame including color coding, refer to section <u>6.2.1 Peptide Candidates Frame</u>.



Below the "Peptide Candidates Frame" is the "Ion Table Frame". Each amino acid is color coded by confidence level (refer to section 6.2.2 Ion Table Frame) 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 strengths of ms/ms peaks that PEAKS has identified 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", refer to section 6.2.3 Spectrum View Frame.

By clicking on the tabs are the bottom of the page, you can view the corresponding survey scan, the spectrum alignment and the error map. For more information, refer to sections 6.2.4 Survey Scan, 6.2.5 Spectrum Alignment Frame and 6.2.6 Error Map.

#### 3.5 RUN PROTEIN IDENTIFICATION

1) With the de novo result selected, click the PEAKS Search toolbar icon i or select "PEAKS Search" from the "Tools" menu.

- 2) Enter the following settings:
  - Error Tolerance:
  - Parent Ion to 20ppm
  - Fragment ion to 0.8 Da.
  - Enzyme: Trypsin
  - PTM: To enter Carboxymethly as a fixed PTM click the "Set PTM..." button and a new window will appear. Select Carboxymethyl from the list on the left and click the arrow on the right that corresponds with "Selected Fixed PTM". Then click OK.

ils.	Database Search	Predefined parameters	Instrument_default	•	Savre as
	Error Tolerance				
la Refinement	Parent ion: 20 ppm •	using monoisotopic mass 👻	Fragment ion: 0.8 D		
ácate Analysis	Enzyme				
Nava	Trypsin		• View Enzyme	New Enz	yme
	Maximum missed cleavages per per	Ride: 1 💠			
KS Search	DTM				
OER Search	F Carboxymethyl			10	Set PTM
Finder					Remove
					Suitch tupe
					Switch (3be
	Maximum allowed variable PTM per	peptide 3 💠			
	Database				
	Select database Database:	Sample DB	-	New	Edt
	Paste seguence Taxa:	all species		Set/New	taca
	General Options				
	Validation with reverse databa	se as decoy			
	Perform on-the-fly preprocess	ng (peak centroiding, charge deconvolutio	n, and deisotope)		
	(If you want to do protein search y	ith different De Novo parameter, please u	ise WorkFlow or select a De Ni	wo node to	start)

Max allowed variable PTM: 3

Note that there is no need to preprocess this data set "on the fly" as we have already preprocessed the data during the data refinement stage.

As with de novo sequencing, to enter Carboxymethyl as a fixed PTM click "Set PTM..." from the right and a new window will appear. Select Carboxymethyl from the list on the left and click the arrow on the right that corresponds with "Selected Fixed PTM". Then click OK.

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 refer to section 7.1 Setting up Protein Identification Parameters.

Click "OK" to commence analysis.

Once the Protein ID file appears in the "Project View Panel", double click on the file and the results will appear in the "Main Processing Window":

The "Peptide View" will appear in the "Main processing" window by default. 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 8 and 9 can both be found under one hit.

The "Peptide Alignment Panel" contains an "Ion Table", "Spectrum View Frame", "Spectrum Alignment Frame", "Survey Scan" and "Error Map". Click on the "Peptide Details" tab. If the spectrum has a de novo hit, then a simple alignment between the original de novo sequence and the peptide found in the database will be shown. At the bottom of the "Peptide Details" panel you will see where the selected peptide matches the protein. For more information about these sections



refer to sections 6.2.2 Ion Table Frame - 6.2.6 Error Map.

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

View	Accession	ID	Mass	Coverage Display	PEAKS(S	core %) ∇	p-va	alue Cover	rage(%)	Query	matched	Descriptio	n				Marke	ec 🛱
tei	🖃 🌑 DB Search																	*
Prol		OVIN 1				8.3	8.25								vine).			
		OVIN 2	69293		8	3.8	1.87	'E-60 3	1.62	;	2	Serum albu	min - Bos	Bos taurus (Bovine).         Sos taurus (Bovine).           os taurus (Bovine).         enase 1 - Saccharomyces cerevi           n         Quality         Start         End         #           0.768         107         120         0.785         457         468				
		EAST 3	36691		. 5	9.5	4.2	E-45 2	:.59		1	Alcohol del	nydrogena	ase 1 - Sacc	haromyce	es cerevi		Ŧ
	•							111										
	Peptides Coverage Tool Box											# Hits	Æ					
		VPQTHYYA	AVAVVK	99.0	1.4E-45	802.9082	2	1603.7993	-0.0	026	1.598	4 0.07	2	0.788	107	120		
		5DANINW	NNLK	98.6	1.4E-45	695.3397	2	1388.6685	0.0	035	2.549	2 0.31	4	0.785	457	468		1
	🖻 🌑 Hit 4 🛛 HS	5TVFDNLP	NPEDR	99.0	1.4E-45	820.8843	2	1639.759	0.0	049	2.977	8 1.42	8	0.787	230	243	2	
	Spectrum 4 HS	5TVFDNLP	NPEDR	97.1	1.4E-45	547.5921	3	1639.759	0.0	045	2.754	4 1.34	7	0.777				
	Spectrum 5 HS	5TVFDNLP	NPEDR	99.0	1.4E-45	820.8843	2	1639.759	0.0	049	2.977	8 1.42	8	0.787				

PEAKS presents a list of proteins, ranked in descending order from highest to lowest score. Clicking on any protein will display the peptides matched to that protein in the bottom pane. In this case the highest scoring protein match is Serotransferrin precursor from bovine. This protein has two matching peptides, which you can see in the "Peptide ID" list when you select the "Peptides" tab. To see exactly where the peptide corresponds to the protein sequence, select the "Coverage" tab. The entire sequence of the protein is shown and the matched parts are underlined in blue and highlighted. In this case the total matched portions accounts for 5.68% of the protein. Place mouse on the blue line, the hit information of that peptide will be shown.



#### 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 result 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.

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- Click the PTM Finder toolbar icon select "PTM Finder" from the "Tools" menu.
- 2) Enter the following settings:
  - Error Tolerance:
    - Parent Ion to 20ppm
    - Fragment ion to 0.8 Da.
  - Enzyme: Trypsin

• PTM: Note that Carboxymethyl is fixed. Deamidation and Oxidation M are variable modifications.

• Max allowed variable PTM: 3

PIMHinder	Predenned	parameters	nstrument_derauit	▼ Save as
Mass Opt	ons			
t Parent M	ass Error Tolerance:	20 ppr	n  Precursor Manada	lass Search Type:
sis	Mass Error Tolerance:	0.8 Da	<ul> <li>Monos</li> </ul>	ocupic 🕜 Average
Enzyme				
Trypsin		-	View Enzyme	New Enzyme
PTM				
V Dean	idation			Set PTM
F Carb	ition M xymethyl			Remove
				Switch type
				Sween cype
Maximum	allowed variable PTM per	peptide 3		
General C	ptions			
Prepro	ess this data 'on the fly'			
Max Misse	d Cleavages: 1 🚔			
Filter Opt	ons			
Filter the	spectra which satisfy the	following condi	ions for use in the PTN	1 search:
De novo	ALC (%) score greater th	ian: 50 %	recommend 50%	
Protein II	peptide score less than	65 %	recommend 65%	

. PEAKS 4 N

• Note that there is no need to preprocess this data set "on the fly" as we have already preprocessed the data during the data refinement stage.

• Parameters can be saved and used for future reference by clicking on the "Save Parameter" button. For more information on setting up PTM Finder parameters refer to section <u>8.1 Setting up PTM Finder Parameters</u>.

3) Click "OK" to commence analysis.

After the PTM Finder search is complete, double click on the result node. The "Peptide View" window will appear:

ysis. hplete, double eptide View"  $\frac{1}{2} \frac{1000}{1000} \frac{11000}{2013} \frac{1000}{2013} \frac{1000}{2000} \frac{1000}{2013} \frac{1000}{2014} \frac{1000}{2013} \frac{1000}{2014} \frac{1000}{2013} \frac{1000}{2014} \frac{1000}{200} \frac$ 

The results will be displayed in the same format as was seen for Protein ID. Recall that the Protein ID search identified spectra 1, 2, 3, 4, 5, 8 and 9. The PTM finder search also displayed spectra 7 with the addition of deamidation on N.

#### 3.7 RUN AN INCHORUS SEARCH

Performing a search with the same data via different search engines is useful both for finding new proteins and confirming others. You can perform an 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, refer to section <u>16.1.3 Search Engine Preferences</u> for more instructions.

1) Click on the Orbisample.mzxml file. Then click the inChorus Search toolbar icon  $\bigotimes$  or select "inChorus Search" from the "Tools" menu.

2) The inChorus Search window will open. "PEAKS Protein ID" box will initially be checked. Enter the following settings:

- Error Tolerance:
  - Parent Ion to 20ppm
  - Fragment ion to 0.8 Da.
- Enzyme: Trypsin

• PTM: Note that Carboxymethyl is fixed. Deamidation and Oxidation M are variable modifications.

• Max allowed variable PTM: 3

Note that there is no need to preprocess this data set "on the fly" as we have already preprocessed the data during the data refinement stage.

- 3) Next, check the "X!Tandem" box.
- 4) Enter the following settings:
  - Database: Sample DB, all species
  - Fragment mass error: 0.8 Da.
  - Complete modifications: Carboxymethly (C)
  - Potential modifications: Oxidation (M), Deamidation (N) and (D)
  - Refinement Specification: Round 1 (none), Round 2 (none)
  - Cleavage site: trypsin, [RK]|{P}
  - FTICR (10 ppm)





- 5) Finally, check the "OMSSA" box.
- 6) Enter the following settings:
- Enzyme: Trypsin
- Max missed cleavages: 3
- Sequence library: Sample DB, all species
- Fixed modifications: carboxymethly C
- $\bullet$  Variable modifications: Deamidation N and D, Oxidation of M
- Precursor mass tolerance (Da): 0.1
- Product mass search type (Da): 0.8
- 7) Click the "OK" button.

inChorus Search		×
Tools	OMSSA	Predefined parameters
PEAKS Protein ID		
SPIDER		OMSSA
XITandem		<u> </u>
💟 OMSSA	HONE SEARCH SITE WAP Search Search S	tatus Browser Download FAQ He
Mascot	Enzyme: Trypsin	Maximum missed cleavages: 1 💌
Sequest		Species to search (ctrl key for multiple selection):
📰 Import Result	Sequence Ebrary:  Sample D8 Taxonomy not supported	All speces Acrohaea Acro
	Hitlist max length: 10 💌	E-value cutoff: 1
	Fixed mods (ctrl key for multiple selection): Carbamylation of K Carbamylation of n-term peptide Carboxyamidomethylation of D Carboxyamidomethylation of H	Variable mods (ctf key for multiple selection): deamdation of N and O carboxymethyl C ortyfulnasion of R periodision of P to perceptutamic acid oxidation of V to perceptutamic acid oxidation of W to formylkynurenin *
	Maximum variable mod combinations searched	d 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 charge	d products: 3 •
	Fraction of product peaks below precursor to	determine +1 precursor: 0.95
	Peak intensity outoff:     O (fraction of most intense)	Number of top intensity peaks in first pass: 6
	Ions to search 1: b 💌	Ions to search 2: Y
	Write to t	the Help Desk   Disclaimer   Privacy statement   Accessit
		OK Cancel Help



When the inChorus search is complete you should see the following new additions in the "Project View" panel. 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, double click on the report that you would like to see in the "Project View" panel.

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

						PEA	AK58 ×						
View	🤤 🧼 🚺 / 1	Displaying peptide hits 1	to 7 out of 7										
oteir	ID	Sequence	PEAKS(Score %)	p-value	M/Z	z	Mr(Calc)	Delta(Mass)	Error(ppm)	RT	Scan	Quality	E
ۍ ع	🖃 🕘 Peptides												1
Vie	Spectrum 1	DNPQTHYYAVAVVK		1.4E-45									
tide	Spectrum 2	TSDANINWNNLK	98.5	4.44E-16	695.34	2	1,388.668	0.004	2.549	0.31	4	0.785	5 (
Pe	Spectrum 3	ANELLINVK	97.7	1.4E-45	507.303	2	1,012.592	0.001	0.784	0.36	5	0.781	I C
ew	🖨 🔵 Hit 27	HSTVFDNLPNPEDR	99.0	1.4E-45	820.884	2	1,639.759	0.005	2.978	1.42	8	0.787	7 C
iV o	O Spectrum 4	HSTVFDNLPNPEDR	96.9	7.77E-16	547.592	3	1,639.759	0.004	2.754	1.34	7	0.777	7 (
Nov	Spectrum 5	HSTVFDNLPNPEDR	99.0	1.4E-45	820.884	2	1,639.759	0.005	2.978	1.42	8	0.787	7 C
De	Spectrum 7	LVN(+.98)ELTEFAK	97.9	1.4E-45	582.81	2	1,163.607	0.001	0.839	1.7	12	0.775	5 (
per	Spectrum 8	SHC(+58.01)IAEVEK	99.0	1.11E-14	537.25	2	1,072.486	-0.001	0.683	1.77	14	0.776	5
ssig	Spectrum 9	TC(+58.01)VADESHAGC(+58.01)EK	99.0	1.4E-45	733.281	2	1,464.55	0.002	1.084	1.87	16	0.787	76.
A N	•			"								•	

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

ID	Sequence	XTANDEM(E-value)	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(ppm)	RT	Scan	Quality	Frag. Mode	#1
🖃 🌑 Peptides												
💭 Spectrum 1	DNPQTHYYAVAVVK										CID, CAD, I.	
O Spectrum 3	ANELLINVK	1.3E-2	507.303	2	1,012.592	0.001	0.784	0.36	5	0.781	CID, CAD, I.	
🖕 🌑 Hit 20	HSTVFDNLPNPEDR	2.4E-9	820.884	2	2 1,639.759	0.005	2.978	1.42	8	0.787	CID, CAD, I.	
Spectrum 4	HSTVFDNLPNPEDR	2.3E-3	547.592	3	1,639.759	0.004	2.754	1.34	7	0.777	CID, CAD, I.	
Spectrum 5	HSTVFDNLPNPEDR	2.4E-9	820.884	2	2 1,639.759	0.005	2.978	1.42	8	0.787	CID, CAD, I.	
Spectrum 7	LVN(+.98)ELTEFAK	6.8E-2	582.81	2	1,163.607	0.001	0.839	1.7	12	0.775	CID, CAD, I.	
Spectrum 8	SHC(+58.01)IAEVEK	3.1E-3	537.25	2	1,072.486	-0.001	0.683	1.77	14	0.776	CID, CAD, I.	
- O Spectrum 9	TC(+58.01)VADESHAGC(+58.01)EK	1.2E-7	733.281	2	2 1,464.55	0.002	1.084	1.87	16	0.787	CID, CAD, I.	

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

											M C	MSSA 6 ×		
1 View	🤤 🧼 🚺 / 1	Displa	aying peptide hits 1	to 4 out o	f 4									
oteir	ID	Sequence	OMSSA(E-value)	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(ppm)	RT	Scan	Quality	Frag. Mode	# Hits	R.
3	🖃 🌑 Peptides													-
Vie	- 💭 Spectrum 1	DNPQTHYYAVAVVK										CID, CAD, IRMP		
fide	Spectrum 2	TSDANINWNNLK	1.04E-4	695.34	2	1,388.668	0.004	2.549	0.31	4	0.785	CID, CAD, IRMP		
Pep	Spectrum 3	ANELLINVK	2.49E-2	507.303	2	1,012.592	0.001	0.784	0.36	5	0.781	CID, CAD, IRMP		
	📥 🌑 Hit 17	HSTVFDNLPNPEDR	7.82E-8	820.884	2	1,639.759	0.005	2.978	1.42	8	0.787	CID, CAD, IRMP		2
	O Spectrum 4	HSTVFDNLPNPEDR	5.46E-2	547.592	3	1,639.759	0.004	2.754	1.34	7	0.777	CID, CAD, IRMP		
	Spectrum 5	HSTVFDNLPNPEDR	7.82E-8	820.884	2	1,639.759	0.005	2.978	1.42	8	0.787	CID, CAD, IRMP		

The inChorus report contains most of the information that is seen in a PEAKS Protein ID results file, refer to section <u>7.2 Protein Identification Results</u> for more details. Click on the "Peptide View" tab.

		-			•		4	INCH	ORUS 9 🗙		
i View	🧼 🧼 🚺 / 1	Displaying peptide hits	1 to 7 out	of 7							
oteir	ID	Sequence	M/Z	InChorus(Score %)	PEAKS(Score %)	OMSSA(E-value)	XTANDEM(E-value)	Z	Mr(Calc)	Delta(Ma	Error(p
3	🖃 🌑 Peptides										*
Vie	- O Spectrum 1	DNPQTHYYAVAVVK									1
tide	Spectrum 2	TSDANINWNNLK	695.34	99.95	98.5	1.04E-4	-	2	1,388.668	0.004	2
Pe	Spectrum 3	ANELLINVK	507.303	99.87	97.7	2.49E-2	1.3E-2	2	1,012.592	0.001	0
	🖨 🔵 Hit 34	HSTVFDNLPNPEDR	820.884	99.98	99.0	7.82E-8	2.4E-9	2	1,639.759	0.005	2
	O Spectrum 4	HSTVFDNLPNPEDR	547.592	99.84	96.9	5.46E-2	2.3E-3	3	1,639.759	0.004	2
	Spectrum 5	HSTVFDNLPNPEDR	820.884	99.98	99.0	7.82E-8	2.4E-9	2	1,639.759	0.005	2
	Spectrum 7	LVN(+.98)ELTEFAK	582.81	99.36	97.9		6.8E-2	2	1,163.607	0.001	0
	Spectrum 8	SHC(+58.01)IAEVEK	537.25	99.72	99.0	-	3.1E-3	2	1,072.486	-0.001	0
	Spectrum 9	TC(+58.01)VADESHAGC(+58.01)EK	733.281	99.98	99.0	-	1.2E-7	2	1,464.55	0.002	1

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, 7-9 X!Tandem found 1, 3-9 and OMSSA confirmed spectra 1-5. In this case, the X!Tandem and OMSSA searches did not generate any extra results that PEAKS did not find but did help to confirm quality matches.

#### 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 section 9 SPIDER Search.

SPIDER Search

Tools

- 1) Click on the Protein ID Result node.
- Next, click the SPIDER Search toolbar icon or select "SPIDER Search" from the "Tools" menu.
- 3) Enter the settings as shown:
- Query Type: Homology Match
- Mass Tolerance: 0.1 Da
- Leucine=Isoleucine
- Lysine=Glutamine
- General Options 5 ≑ . Data Refinement Query Type: 💿 Tag Match Homology Match Report Top: . Replicate Analysis Mass Error Tolerance 🗸 Lysine=Glutamin 0.1 🔽 Leucine=Isoleucine Mass Tolerance: Da . De Novo . PEAKS Search ртм Deamidation
   Oxidation M
   F Carboxymethyl Set PTM... ✓ SPIDER Search Remove . PTM Finder Switch type Maximum allowed variable PTM per peptide 3 Filter Use the spectra which satisfy the following conditions for use in the SPIDER search De novo (ALC %) score greater than: 50 recommend 50% Protein ID peptide score less than: 65 recommend 65% OK Cancel Help

Predefined parameters default

▼ Save as...

• PTM: Carboxymethyl as fixed. Deamidation and Oxidation M as variable modifications.

- Max allowed variable PTM: 3
- De Novo (ALC%) score greater than: 50
- Protein ID peptide score less than: 65

Parameters can be saved and used for future reference by clicking on the "Save Parameter" button. For more information on setting up SPIDER Search parameters refer to section <u>9.1 Setting up SPIDER Parameters</u>. Click "OK" to commence analysis.

After the SPIDER search has completed, double click on the SPIDER results node. The "Peptide View" window will appear. The format is similar to what was seen in the results of a Protein ID search with the addition of a few columns of information such as Homolog used, PEAKS result used and SPIDER score. Note that all spectra can now be identified by the SPIDER search. Spectrum 6 is finally identified.

Clicking on the "Peptide Details" tab will display the protein with its matched peptides in red. SPIDER will also display a reconstructed sequence. Refer to section <u>9.2 SPIDER Results View</u> for more information.

Click on the "Protein View" tab, select a result and then click on the "Coverage" tab to see where the SPIDER results overlap with the protein sequence. Note that mutated sequences identified by SPIDER are shown in red while exact matches found by PEAKS protein ID or SPIDER are in blue.



# Chapter

#### 4 LOADING SPECTRUM DATA

#### 4.1 DATA FORMATS

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:

- .RAW files from Thermo Fisher Scientific instruments
- .D directories from Agilent QTOF instruments
- .BAF, .YEP and folders of .FID files from Bruker instruments
- .RAW directories from Waters QTOF instruments
- .WIFF files from AB/Sciex QSTAR and QTRAP instruments
- .RUN folders from Shimadzu instruments
- mzXML format
- mzData format
- mzML format
- .DTA files
- .MGF files
- .PKL files
- .XMS files from Varian instruments
- PEAKS 5.1 project directories
- PEAKS 5 project directories
- .ANZ the zip compressed XML based file format associated with PEAKS 4.5 and earlier

#### 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 scans and corresponding 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.

#### 4.2.1 PEAKS PROJECT CONVERTER

#### Convert from PEAKS 5.1 to 5.2

Projects created in PEAKS 5.1 can be opened in PEAKS 5.2. To convert the project to a PEAKS 5.2 project, open the PEAKS 5.1 project in PEAKS 5.2. The project will be recognized as a PEAKS project from an older version. A window will appear stating, "This project was created by an older version of PEAKS. Do you want to convert it now?" Choose Yes. The following dialog box will appear.

In the new window choose		er	23
the converted project name			
and location. If a location is	Converted Projects	New Project 1_version5_2	
not chosen, the PEAKS 5.2	Project Location	C:\PeaksStudio5.2l.\derbyServer\serverDB	Browse
derby server is the default		- · · - · · · · · · · · · · · · · · · ·	
location. A new version of		50%	Start
the project will be created at			
the new location and the old			

project will be preserved in its original location.

#### **Convert from PEAKS Studio 5.0 to 5.2**

A direct conversion from PEAKS 5.0 projects to 5.2 projects is only supported in command line. PEAKS provides a straight-forward project conversion tool that will allow users to quickly convert existing 5.0 projects into PEAKS 5.2 format. The resulting PEAKS 5.2 project can be loaded PEAKS Studio 5.2. Users comfortable with command line, will find the tool very easy. This section will cater to those unfamiliar with command line, but who wish to convert their projects promptly.

There are two methods that can be used to generate a PEAKS 5.2 project from an existing 5.0 project.

1) The first method converts a 5.0 project and stores the 5.2 project in the same directory as the original file, with the addition of "52" to the end of the file. Just enter ProjectConverter followed by the PEAKS 5.0 project location and name. For example, if the project is found in your C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project folder, enter

#### ProjectConverter "C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project"

This will produce a new 5.2 project in this same directory, with the name My5.0Project.52.

The quotation marks ("") surrounding the location are not necessary in this case, but are necessary if your path contains any spaces, such would be found in use of "C:/My Documents/Projects...".

2) To produce a new file in a different directory, the only change needed is the addition of a blank space, followed by entering the output directory, such as "C:\PeaksStudio5.2\derbyServer\serverDB\My5.2Project"

Thus the line will look like (in one line)

ProjectConverter "C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project" "C:\PeaksStudio5.2\derbyServer\serverDB\My5.2Project"

If the new directory does not already exist, the program will create the directory for you. If the directory already exists, entering a dash and the letter '-f' before the original project will force the ProjectConverter to overwrite any existing projects in this location.

For example:

#### ProjectConverter -f "C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project"

Or

ProjectConverter -f "C:\PeaksStudio5.0\derbyServer\serverDB\My5.0Project" "C:\PeaksStudio5.2\derbyServer\serverDB\My5.2Project"

You are now ready to call up these projects into PEAKS 5.2.

#### 4.2.2 AGILENT DATA

PEAKS 5.2 can load native data from Agilent QTOF, provided that MassHunter software is installed on the same computer.

Agilent Ion Traps produce a .d folder which contains all data associated with that data set. The spectral data is contained in the .yep file. CompassXport can be used to read the .yep file and convert it to an mzXML file inside the .d folder. This file is then loaded into PEAKS. CompassXport must be installed on the same computer as PEAKS to perform this conversion automatically.

#### 4.2.3 APPLIED BIOSYSTEMS (WIFF) 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 5.2. 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.

#### 4.2.4 APPLIED BIOSYSTEMS (4700/4800) 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 (we require that there is no firewall or proxy) 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.

#### 4.2.5 BRUKER DATA

PEAKS 5.2 can load data from Bruker mass spectrometers provided that the CompassXport software is installed on the same computer. If loading fid files, select the top level folder to load them all at once (make sure the merge option is set in the Bruker instrument preferences refer to section <u>16.1.2.2 Bruker (.yep/baf, fid)</u>). CompassXport 3.0 is readily available on the Bruker Daltonics web site. Autoflex and Ultraflex Tof/Tof instrument data files may require CompassXport 1.3. If spectra cannott be loaded into PEAKS with CompassXport 3.0 contact Customer Support. You may need to contact your Bruker representative to obtain CompassXport 1.3.

#### 4.2.6 SHIMADZU DATA

Shimadzu mass spectrometer data can be loaded, provided that the Shimadzu software is installed on the same computer as PEAKS 5.2.

#### 4.2.7 THERMO DATA

RAW data from Thermo Fisher Scientific mass spectrometers can be loaded, provided that the XCalibur software or the Thermo MSFileReader package is installed on the same computer as PEAKS 5.2.

#### 4.2.8 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 spectral data in the viewed chromatogram is converted to the .pkl format. If you are viewing a single spectrum and choose to convert the data, only the viewed spectra will be converted.

Importing raw data that has not been preprocessed will produce better results when using the preprocessing options native to PEAKS.

#### 4.2.9 WATERS/MICROMASS (MASSLYNX) DATA

PEAKS 5.2 can import RAW data from Waters/MicroMass QTOF instruments, provided that MassLynx 4.1 software is installed on the same computer. For MassLynx 4.0 users, a utility called wolf.exe (originally created as

part of the Sashimi Project) can be used to convert the raw files to mzXML format in command line. If you encounter difficulty loading Waters data into PEAKS you may require a different version of wolf, please visit:

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

#### 4.3 CREATE A NEW PROJECT

- 1) To create a new project, select "New Project" from the file menu or using the "New project" icon **the** toolbar. A new window will appear.
- Create a name for your project. The default project location will be automatically displayed. Click "Browse" to select where you would like to save the project.
- 3) To add data files to sample 1, click the "Add data files" button. Browse for the data file and click the "Open" button. Once the data file appears, select the "Instrument Vendor" and "Instrument type" that was used to generate the experimental data from the drop-down lists. Selecting the "All Instruments" option from the "Instrument Vendor" drop-down list will display some general instrument types in the "Instrument Type" drop-down list. If no fragmentation mode is specified in the instrument type name (in brackets), the default setting is CID. If you would like to apply the same instrument configuration to all of the files in the sample, or to all of the samples in the project click on the "whole sample" button or "whole project" button, respectively.
- 4) To add another sample, click on the "Add Sample" button. To add a data file to Sample 2 click on the "Add data files" button. Select the instrument vendor and type from the drop-down menus unless you had previously applied the instrument configuration to the whole project in step 3.
- 5) To use the new Replicate Analysis feature you can set up to 3 samples to be replicates of the same experiment. To do this, select the sample node that is the first replicate, click the replicate check box and choose '1' from the drop down menu. Follow this process for the second and third replicate.
- 6) To delete a sample or data file, select the appropriate node (sample or data file) and click the "Delete" button. You can also change the order of the samples within a project or data files within a sample using the "Up" and "Down" buttons.
- 7) Click the "OK" button once all data files and samples are added to the project.
- 8) The project will appear in the "Project View" window. The outlined " <sup>C</sup> " symbol indicates that the file is still loading. The solid " <sup>C</sup> " symbol indicates that the file has finished loading .

#### 4.4 ADD DATA TO AN EXISTING PROJECT

Select "Add data" from the file menu or "" from the task bar. The original project window will open. You can add more files to an existing sample or create additional samples. You will need to select the instrument vendor type. For more information on adding files/samples or setting up the instrument configuration refer to section <u>4.3</u> Create a New Project.

#### 4.5 OPEN / CLOSE A PROJECT

To open a stored project, select "Open Project" or "Open Recent Project" from the file menu or "—" from the task bar. To close a project that you are working on, select "Close Project" from the file menu or "…" from the task bar.

#### 4.6 CHANGE THE DEFAULT LOCATION OF PROJECTS

Projects are saved in the location that is listed in your "Preferences" window. To modify your preferences, select the "Preferences" toolbar icon "O" 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. Click on the "Browse" buttons to change the default output directory.

#### 4.7 ORIENTING YOURSELF

#### 4.7.1 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 you want to analyze.



Make sure the data file to be analyzed is selected. If you select a project, all of the samples will be analyzed and displayed in a single result file. If you select a sample, all of the files within that sample will be analyzed and displayed in a single result file.

Note: Any features that cannot be performed when a specific node is selected will be grayed out. In the example below, you cannot run the PTM finder when the raw file is highlighted; you must have a PEAKS Protein ID result selected in order to execute the PTM finder.

#### 4.7.2 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.

Tasks Running Info	Properties	
Selection Details: Orb	iSample.mzXML	•
Total MSI Spectra Total MSI/MS Spectra Ion Source Fragmentation Mode MS Scan Mode MS/MS Scan Mode MS Scan Centroid MSI/MS Scan Centroid	7 9 ESI(nano-spray) CID, CAD, IRMPD (y and b ions) FT-ICR/Orbitrap Linear Ion Trap false false	II
		Ŧ

#### 4.7.3 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 along 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 scan is available, it will be displayed below the corresponding MS1 scan.



To zoom either on the X or Y axes, select the "2X" or "2Y" buttons, respectively. To zoom in further, click and drag the mouse arrow to the side. To zoom into the intensity axis use slide the scroll bar on the left hand side.

Selecting the "1:1" button will bring you back to the original image ratio 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 bottom right hand side (see the box highlighted in red below). Features above an intensity threshold will have their m/z displayed above them.



The MS/MS tab gives detailed information about each tandem spectrum:

Each of the spectra in the data file will be listed in the left most panel under the name of the data file. The precursor mass of the fragment is displayed followed by the charge. If data preprocessing was executed, the processed data will appear. Clicking on the '+' beside the fragment will display the raw data node as the second entry in the tree. Clicking on one of the spectra will display any results that have been generated for that spectrum in the top right hand panel.





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 it you will find the corresponding MS spectra located in the "Survey" tab.

# Chapter

#### 5 **DATA REFINEMENT**

Since mass spectrometry data most often contains noise and redundant data, it is logical 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.

#### 5.1 RUN DATA REFINEMENT

Data refinement is executed on each fraction file. You can run data refinement by selecting fraction, sample or project on the project tree and all the fraction(s) under the selected node will be refined. To begin the refinement of data from a whole MS/MS: D.

1) In the "Project View Frame", select a node containing the data file(s) that you wish to refine.

2) Click the Data Refine toolbar icon Or Select "Data Refine" from the "Tools" menu.

The Data refinement options window will appear:

3) Choose the data refinement tools you wish to use by selecting the checkbox next to each refinement option. See the information below to help you decide on appropriate refinement parameters.

#### 5.2 DATA REFINEMENT PARAMETERS

#### 5.2.1 MERGING SCANS

In Data Dependent Acquisition (DDA) mode, a mass spectrometer will often produce several tandem (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 or ppm. For retention time, we use the units that are recorded in the data file (usually minutes or seconds).

🕺 Data Refinement	×
Tools	MS/MS Data Refinement
	✓ Merge Scans
✓ Data Refinement	Retention time window (for raw files only): 1 min
. Replicate Analysis	Precursor m/z error tolerance: 0.01 💿 Da 💿 ppm
. De Novo	Correct Precursor Mass
	Correct Precursor Charge States
. PEAKS Search	Min charge: 1 🗮 Max charge: 4 🚔
. SPIDER Search	
	M Filter Scans
. PTM Finder	Only keep scans satisfying:
	✓ Precursor mass between 350 and 6000 Da
	Quality value greater than 0.65 (suggest 0.65)
	Data Preprocess (peak centroiding, charge deconvolution, and deisotope)
	💿 no, already done 💿 yes 💿 no
	OK Cancel Help

#### 5.2.2 MASS CORRECTION

In many cases, Thermo Fisher Scientific LTQ-FT and LTQ-Orbitrap instruments record a fragmented precursor mass, which is offset from the correct mono-isotopic mass of the precursor by one or more isotopes. As a result, a significant number of spectra will not be identified unless. PEAKS provides a mass correction function to accurately determine the parent charge state and mono-isotopic mass for high resolution data.

#### 5.2.3 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 high enough resolution to allow for this type of analysis. Thus, PEAKS analyses the MS/MS data to determine whether it's charged 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. The dropdown boxes will allow you to set a range of charge values. Only spectra that fit in this range will be considered for analysis.

#### 5.2.4 FILTERING MS/MS SCANS

Scans of contaminants and electrical noise should not be included in your 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 filtering these low quality MS/MS scans. The fields located under "Filter Scans" will allow you to define ranges of retention time and m/z ratio. Only peaks located within these defined ranges will be considered for further analysis. Additionally, PEAKS can examine 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 quality score threshold (a value from 0 to 1) for accepting a given scan. We recommend a quality score threshold of 0.65.

#### 5.2.5 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 we recommend having the original spectral data available in the event you need to refer to it at a later time.

To see how your data has 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

In the "Project View Frame", select the data file(s) or project containing the spectra that you wish to sequence by auto de novo. Note that users can run a database search on a fraction or sample level by selecting the fraction node or sample node respectively.

Click the automatic de novo toolbar icon 4 Or Select "auto de novo" from the "Tools" menu. The auto de novo parameters dialogue window will appear.

You now have the option of changing any of the de novo parameters.

# 6.1.1 MASS OPTIONS

Parent mass error tolerance: This determines the degree of random and systematic experimental error on the parent/precursor ion that PEAKS will allow for during the analysis. Since the instrument used for the experiment has been previously selected, PEAKS will provide the suggested error tolerances for the selected instrument. Custom error tolerances can be defined by entering a tolerance in the textbox and

Tools	De Novo	Predefined parame	eters Instrum	ent_default	•	Save as
	Error Tolerance	)				
. Data Refinement	Parent ion: 20	ppm 👻	Fragment	ion: 0.8 Da		
. Replicate Analysis	Enzyme					
✓ De Novo	Trypsin		•]	View Enzyme	New Enzy	/me
. PEAKS Search . SPIDER Search	PTM Carboxymethy	1				Set PTM Remove
, Privi Filider	Maximum allowed v	ariable PTM per peptide [	3 🜩			Switch type
	General Options	s s data on-the-fly (peak ce 🚔 candidates per spect	ntroiding, char rum	ge deconvolution an	d deisotope	∍)
				OK Car	ncel	Help

choosing the desired units from the drop-down list. PEAKS provides two options for the value of mass error tolerance: Da and ppm.

Fragment mass error tolerance: This determines the degree of random and systematic experimental error on the fragment/daughter ion that PEAKS will allow for during the analysis. Again, PEAKS will provide suggested error tolerances based on the instrument selected. Custom error tolerances can be defined by entering a tolerance in the textbox and choosing the desired units from the drop-down list.

# 6.1.2 ENZYME OPTIONS

Enzyme: This informs PEAKS as to what type of enzyme was used to digest the sample. Choose from a drop-down list of enzymes, or if your enzyme (or combination of enzymes) is not in the list, click the "New Enzymes" button. You can then input the name of the new enzyme and define custom cleavage rules/sites and select if you would like to allow up to one end of a peptide to disobey the cleavage rule.

# 6.1.3 PTM OPTIONS

Selecting fixed and variable PTMs: The "PTM Options" list informs PEAKS as to what types of post-translational modifications to include in its analysis. To view additional modifications, select the "Show Unimod" box in the PTM dialog 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 particular PTM. To select a PTM as Fixed or Variable, click the PTM and click the arrow beside the Fixed Modification or Variable Modification box. If you move 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.

# 6.1.4 GENERAL OPTIONS

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

Preprocess this data "on the fly": PEAKS has its own built-in preprocessor for removing noise, centroiding and deconvolution. Check this box to turn preprocessing on

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

After setting up the desired parameters, you can save them for future use. Click the "Save Parameters" button at the top of the window, and define a name for these preferences for future use/reference when prompted. Any parameters that are saved will be available in the drop-down list at the top of the window. To examine the contents, select a saved parameters file and the parameters boxes will be automatically populated.

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

# 6.2 DE NOVO SEQUENCING RESULTS

Once de novo sequencing is completed, the following window will appear:

# 6.2.1 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 (Refer to section <u>5.2.4</u> Filtering MS/MS Scans for more information on how the quality score is generated).



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 sequence of the peptide (including 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.
Rank	The sequences for a particular spectrum (ID) as sorted by score (TLC).
m/z	The measured mass/charge value, in Daltons, for the peptide.
Z	The calculated charge value for the peptide.
Mass	Calculated using the measured m/z and 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.
Scans	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.
Frag. Mode	Mode that the fragmentation step was performed in.

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

The columns themselves are customizable. To change the columns that are displayed click on the "," button.

# **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 Ta	g										8
Sequence Tag	NNP	[128.	06]TH	IYYAV	AVVK						
Score Threshold								0			
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0

 Sequence Tag
 X

 Sequence Tag
 NNPQTHYYAVAVVK

 Score Threshold
 0.0

 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: H(+15.99)ATVFDNLNPPEDR

The +15.99 in brackets refers to a position where a modification may have occurred. If you forget what possible modifications you specified before running de novo, click the "Properties" tab.

The fixed modification is set to Oxidation HW 15.99. In the sequence above, the modification has been made to the H. The colors assigned to the (+15.99) 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:	Trypsin(f:	Oxidation HW)
-----------------	------------	---------------

Parent Mass Error Tolerance	0.1 Da
Fragment Mass Error Tolerance	0.6 Da
Enzyme	Trypsin
PTMS Fixed Modifications Name Oxidation HW	Delta 15.994915
Max variable PTM per peptide	2
Report # peptides	5
Preprocess data	false
Data Refine dependencies	1

# 6.2.2 ION TABLE FRAME

#	Immonium	Ь	b-H2O	a	с	Seq	У	y-H2O	z	z'	y (2+)	#
1	88.04	116.03	98.02	88.04	133.06	D	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	Т	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, refer to section 16.1.4 Ion Editor Preferences.

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.

# 6.2.3 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.

The "Intensity threshold" check box provides an option to display lower intensity peaks in the "Spectrum View Frame"

More options are provide for view the spectrum.

- Ion Display: Immonium, b, b-H2O, y, y-H2O, y (2+), Internal Ion
- m/z Display: show m/z with ions, show m/z on peaks without ions
- Intensity Display: High, Medium, Low

To zoom either on the X or Y axes, select the "2X" or "2Y" buttons, respectively and then use your mouse to move navigate the graph. Selecting the "1:1" button will restore settings the default view which displays the entire spectrum.

The ErrTol is used to adjust the error tolerance to view the display of matched ions.

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 allows you to zoom into the intensity axis of the peaks.

# 6.2.4 SURVEY SCAN

The "Survey" tab displays the precursor ion spectrum. The buttons that appear in this section are identical to those explained above in the "Spectrum View Frame" section.



# 6.2.5 SPECTRUM ALIGNMENT FRAME

The "Alignment" tab displays the entire spectrum and is used as a tool to help us navigate the spectrum view frame. The blue bar along the horizontal m/z axis of the alignment view indicates the range of the spectrum view in



the Spectrum View Frame. This frame displays 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.

## 6.2.6 ERROR MAP

Click on the "Error Map" tab. The m/z ratio is displayed on the x-axis and the error is listed on the y-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.

# 6.3 AUTO DE NOVO ON A SINGLE SPECTRUM.

Auto de novo sequencing on a single spectra can be performed on the MS/MS view as displayed below.



# 6.4 MANUAL DE NOVO SEQUENCING

PEAKS 5.2 provides a set of tools to help you manually sequence a peptide, using graphic cues from the spectrum.



6.4.1 MANUAL DE NOVO GRAPHICAL USER INTERFACE

The figure below shows the main panels related to manual de novo. The five main panels are indicated in the figure below:

The panels are briefly described below:

The "**Result Panel**" shows all the sequencing results. The results of manual de novo are listed in the sub-tree with root "Manual de novo".

The "**Spectrum View Panel**" shows a graphical representation of the spectrum, the peaks in the spectrum, the userselected peaks and assigned ions. Users can pick a peak on the panel and assign ions or tags to it in Manual de novo.

The "**Alignment View Panel**" shows how the proposed ions assigned in manual de novo 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. The "**Ion Table Panel**" shows the proposed ions with their corresponding masses. The default Ion Table will display immonium, b, b-H2O, a, c, y, y-H2O, z, z' and y(2+) ions.

The "**Tag Panel**" will appear when you search tags or ions in the spectrum. You can select the tags in the list using the "Select" button. Clicking "Apply" will add the selected tags to the sequence candidate.

# To create a new peptide candidate for sequencing:

Highlight the m/z value in the "Results Panel" and right click. This will bring up a pop-up menu.



Select "New Candidate for Manual De Novo" from the pop-up menu. A new candidate will be created under the 'Manual de novo' heading. The new candidate will not have been sequenced, so it will be represented by the mass of the peptide less the mass of water (see an example below).

▶ 802.9 	▶ 802.9082 2 (id=1) 											
#	Immonium	Ь	Ь-Н2О	а	с	Seq	У	y-H2O	z	z'	y (2+)	#
1						[1585.80]						1

Note: The popup menu will not be accessible if you have highlighted any of the results in the "Results Panel".

# 6.4.2 MANUAL DE NOVO OPERATIONS

# Note: All operations occur in the Spectrum View Frame of the Main Processing Window.

When the mouse cursor is placed in the Spectrum View Frame, a green (by default) arrow follows the movement of the mouse. This is the Position Bar and it is used as a cursor for all manual de novo operations. The cursor's position on the m/z scale is enumerated on the top of the Position Bar.



# Selecting a peak

To select a peak, simply click on it. A blue (by default) arrow, called the Freeze Bar, indicates the selected peak.

Alternatively an ion peak can be selected by clicking on its corresponding cell in the Ion Table.

### Measure the m/z difference between two PEAKS

Select a peak (blue arrow) with the Freeze Bar and move the mouse to the left or right. Hold the Position Bar (green arrow) above another peak. The number in the Position Bar is the difference between the two PEAKS (in the example below the difference is 80.99109).



### **Deselect** a peak

Double click anywhere in the Spectrum View Frame to deselect a peak.

#### Zoom in on part of the spectrum

In the Spectrum View Frame, click and drag the mouse horizontally. The selected area will be enhanced and shown in the Spectrum View

Frame. Click on the magnifying tool "<sup>SS</sup>" to display a panel with the magnified spectrum. The peak which is selected by the Position bar will be highlighted in the zoomed panel in green.

Click on the magnifying tool again to return to the default view.



### Setting/removing ions to/from a peak

Select a peak, and then right click the mouse anywhere in the Spectrum View Frame. Select "Set y-ion" from the pop-up menu to designate the peak as a y ion, or "Set b-ion" from the pop-up menu to designate the peak as a b ion. Click on "Remove ion" to remove the ion that you have previously set.

Select "Set other ions" from the pop-up menu to view the "Ion Editor" dialog box. The Ion Editor dialogue allows you to add or remove ion designations to/from a peak. Select either "C Term Ion" or "N Term Ion" to see the C and N terminal ions respectively. Then select an ion from the ion list and press the "Add" button to add it to the selected ion list. Remove an ion from the selected ion list by selecting it and pressing the "Remove" button. Click "Apply" to apply the changes to the selected peak.



After setting an ion, both the alignment view and the peptide sequence candidate name (as displayed in the peptide candidate frame) will change to reflect the mass remaining to be sequenced on either side of the ion.





Note that the manual de novo candidate information is updated in the results panel, ion table and alignment view panel. The selected ions are also highlighted in the spectrum view panel. After setting two ions, PEAKS 5.2 will estimate the residue found between them (if a residue corresponds closely to the mass difference). The peptide sequence candidate name (as displayed in the peptide candidate frame) will change to show the residue and the mass remaining to be sequenced on either side of the residue.



### Searching the left or right side of the spectrum for the first/last y or b ion (Search a sequence tag)

Select a peak, and then right click the mouse anywhere in the Spectrum View Frame to trigger the popup menu. From the menu, select either "Left tags" or "Right tags". PEAKS will select the appropriate terminal tags and show them in the "Tag Panel" frame (see below).





If you have made an error in your sequencing it is possible to undo the change. With the Peptide candidate still selected in the "Results panel", right click the mouse and select "Undo" to return to the previous peptide sequence. You can use this button multiple times to return to earlier stages in your edit. You can test the suitability of a tag by highlighting it in the "Searched Tags" panel; the corresponding information for the tag will be shown in the Spectrum View panel, the Ion table and the Alignment frame panel. You can insert one or more tags by highlighting the desired tags, clicking "Select" to move them into the "Selected Tags" panel and then clicking "Apply". Press the "Cancel" button at any time to exit the search and discard any changes.



### **Redoing an edit**

With the Peptide candidate still selected in the "Results panel", right click the mouse and select "Redo" if you have undone one too many changes. You can click this button multiple times to return to later stages in your edit.

# Chapter

# 7 DATABASE SEARCH

# 7.1 SETTING UP PROTEIN IDENTIFICATION PARAMETERS

In the "Project View Frame", select the data file(s) or project containing the spectra that you wish to identify using database search. Note that we can run database search on a fraction or sample level by selecting the fraction node or sample node respectively.

Click the PEAKS Protein ID toolbar icon or Select "PEAKS Protein ID" from the "Tools" menu. The Protein Identification Parameters dialogue window will appear:

You now have the	PEAKS Search	Σ	3
option to change any of the protein	Tools	Database Search Predefined parameters Instrument_default  Save as	
identification search parameters.	. Data Refinement . Replicate Analysis . De Novo ✓ PEAKS Search . SPIDER Search . PTM Finder	Error Tolerance         Parent ion: 20 ppm using monoisotopic mass register to the start of	
		UK Cancel Help	

# 7.1.1 MASS OPTIONS

Parent and Fragment mass error tolerance: refer to section 6.1.1 Mass Options.

Precursor mass search type: If the precursor mass is monoisotopic value, select monoisotopic from the dropdown list next to "using". Otherwise, select average.

# 7.1.2 ENZYME OPTIONS

Enzyme: refer to section <u>6.1.2 Enzyme Options</u>.

Maximum missed cleavages: determines the greatest number of missed cleavages to allow.

# 7.1.3 PTM OPTIONS

PTM options: refer to section 6.1.3 PTM Options.

Max variable PTM per peptide: refer to section 6.1.3 PTM Options.

# 7.1.4 DATABASE OPTIONS

Database: From the drop-down list select one of the FASTA databases configured in PEAKS. To edit an existing database, click the "Edit..." button. If the desired database is not in this list, click the "New..." button.

Note that you can also set up a new database in the "Database Configuration" window. The configuration window is the only place where you have the option deleting databases which you have created. For more information on setting up new databases refer to section <u>16.2.3 Database Configuration</u>.

Set/View Taxa: 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/View 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 sequence: If you already know the sequence of the protein(s) you are looking for, select "Paste sequence" 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.

# 7.1.5 GENERAL OPTIONS

Validate with reverse database as decoy: This executes a Protein ID search on a database that is physically reversed. The result gives an indication of how many random matches we should expect.

Perform on-the-fly processing: PEAKS has its own built-in processor for removing noise, centroiding and deconvolution. Check this box to turn processing on.

After setting up the desired parameters, we can save them for future use. Click the "Save Parameters" button, and define name for these preferences for future use/reference, when prompted. Any parameters that you save will be available in the drop-down list at the top of the window. To examine the contents, select a saved parameters file and parameters boxes will be populated.

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

# 7.2.1 PEPTIDE VIEW

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

						1	PEA	KS8 ×						
h View		Image:												
oteir	ID		Sequence	PEAKS(Score %)	p-value	M/Z	z	Mr(Calc)	Delta(Mass)	Error(ppm)	RT	Scan	Quality	₽
l à	<b>-</b>	Peptides 🕽												
š		💭 Spectrum 1	DNPQTHYYAVAVVK	99.0	1.4E-45	802.908		1,603.799	-0.003		0.07		0.788	
tide		Spectrum 2	TSDANINWNNLK	98.5	4.44E-16	695.34	2	1,388.668	0.004	2.549	0.31	4	0.785	(
Pe -		Spectrum 3	ANELLINVK	97.7	1.4E-45	507.303	2	1,012.592	0.001	0.784	0.36	5	0.781	¢.
ŝ		🗕 🔘 Hit 27	HSTVFDNLPNPEDR	99.0	1.4E-45	820.884	2	1,639.759	0.005	2.978	1.42	8	0.787	C
i N		Spectrum 4	HSTVFDNLPNPEDR	96.9	7.77E-16	547.592	3	1,639.759	0.004	2.754	1.34	7	0.777	C
No.		Spectrum 5	HSTVFDNLPNPEDR	99.0	1.4E-45	820.884	2	1,639.759	0.005	2.978	1.42	8	0.787	C
De		Spectrum 7	LVN(+.98)ELTEFAK	97.9	1.4E-45	582.81	2	1,163.607	0.001	0.839	1.7	12	0.775	ć
led		Spectrum 8	SHC(+58.01)IAEVEK	99.0	1.11E-14	537.25	2	1,072.486	-0.001	0.683	1.77	14	0.776	¢
ssig		Spectrum 9	TC(+58.01)VADESHAGC(+58.01)EK	99.0	1.4E-45	733.281	2	1,464.55	0.002	1.084	1.87	16	0.787	¢
a Ne	•				11								Þ	

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". 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. PTMs are listed in [square brackets].
PEAKS(Score %)	PEAKS' probability score.
p-value	The p-value of the peptide
M/Z	The measured mass/charge value, in Daltons, for the peptide.
Z	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.
Frag. Mode	Mode that the fragmentation step was performed in.
# Hits	Number of hits corresponding to peptides with multiple spectra.

The columns themselves can be customized. To change the columns displayed click on the "," button.

# **Peptide Alignment**

Click on the "Peptide Align" window. Similar to the de novo results window, the "Ion Table" displays proposed ions with their corresponding masses. Below the "Ion Table" is the 'Spectrum View Frame" and below this is the "Error Map" which displays confidence 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.

The "Spectrum View Frame" located below the "Ion Table" 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 onto the X or Y axes, select the "2X" or "2Y" buttons, respectively. Then use your mouse to navigate the graph. Selecting the "1:1" button will restore settings the default view which displays the entire spectrum.

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 allows you to zoom into the intensity axis of the peaks.

Clicking the "Alignment"	Info Survey Alignment	Error Map					
tab will display the	(%) RH5		FD N P	N L	LND	PNPEDR FVTSH	bMax yMax
"Spectrum Alignment	Intens						m/z
Frame". This frame will		200 400	600	800	1000	1200 1400	1600

always display the entire spectrum and is used as a tool to help us navigate the spectrum view frame. The blue bar

along the horizontal m/z axis of the alignment view indicates the range of the spectrum view in the Spectrum View Frame. This frame displays 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.

Clicking on the "Survey" tab will display the corresponding precursor ion spectrum. The buttons that appear in this section are identical to those explained above in the "Spectrum View Frame" section. Click on the "Error Map" tab. The m/z ratio is displayed on the x-axis and the error is listed on the y-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.

# **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 selected in the "Peptide View" window. If more than one protein matches a single peptide, you will be able to select and view these additional proteins using the drop-down 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.

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. The matched peptides will be shown in red if you have performed a SPIDER search which is discussed in the next chapter.

# 7.2.2 PROTEIN VIEW

Clicking on the "Protein View" tab will produce the following window:

view	Accession		ID	Mass Covera	ige Display	PEAKS(Score	%)	p-value	Coverage(%)	Query matche	d De	scription					R.
ein	🖃 🌑 DB Search																-
Prof	💭 Q29443 TF	RFE_BOVIN		77753.200		98.3		8.25E-135	5.68		Ser	otransfe	rrin - Bos ta	urus (Bov	ine).		
ŝ	P02769 AL	BU_BOVIN	2	69293.550		83.8		1.87E-60	3.62	2	Ser	um album	nin - Bos tau	irus (Bovir	ne).		
e C		H1_YEAST	3	36691.957		59.5		4.2E-45	2.59	1	Alc	ohol dehy	/drogenase	1 - Sacch	aromyce:	s cerevisi.	
Jovo View Pep	Peptides Coverage	Tool Box															+
De	ID	Sequence		PEAKS(Score %)	) p-value	M/Z	Z	Mr(Calc)	Delta(Mass)	Error(ppm)	RT	Scan	Quality	Start	End	# Hits	R\$
Per	Spectrum 1	DNPQTHYYA	VAV\	к 99.0	1.4E-45	5 802.9082	2	1603.7993	-0.0026	1.5984	0.07	2	0.788	107	120		
ssig	Spectrum 2	TSDANINWN	INLK	98.6	1.4E-45	695.3397	2	1388.6685	0.0035	2.5492	0.31	4	0.785	457	468		1
A 3	🖕 🌑 Hit 4	HSTVFDNLPN	<b>IPED</b>	R 99.0	1.4E-45	5 820.8843	2	1639.759	0.0049	2.9778	1.42	8	0.787	230	243	2	
Ś	O Spectr	HSTVFDNLPN	IPED	R 97.1	1.4E-45	5 547.5921	3	1639.759	0.0045	2.7544	1.34	7	0.777				
ò	Spectr	HSTVFDNLPN	IPED	R 99.0	1.4E-45	5 820.8843	2	1639.759	0.0049	2.9778	1.42	8	0.787				

The "Protein View" collects all the peptide identifications together, summarizing 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 here are organized to best enable us to evaluate the results at the protein level.

tide Ali	ign Peptide Deta	ils				
ct pept	ides for display:	229443 TRFE_BOV	'IN	*		
						~
	De Novo:DNPQ	THYYAVAVVK				
	Real: DNPQ	THYYAVAVVK				
	1111	11111111111				
	RSD (h=0,1,2,3	):				
	0000					
	DB peptides show	n in blue				
	MRPAVRALLA	CAVEGECEAD	PERTVRWCTI	STHEANKCAS	FRENVERILE	
.01						=
	SGPFVSCVKK	TSHMDCIKAI	SNNEADAVTL	DGGLVYEAGL	KPNNLKPVVA	
201						
	EFHGTKDNPQ	THYYAVAVVK	KDTDFKLNEL	RGKKSCHTGL	GRSAGWNIPM	
101						
	AKLYKELPDP	QESIQRAAAN	FFSASCVPCA	DQSSFPKLCQ	LCAGKGTDKC	
101						
	ACSNHEPYFG	YSGAFKCLME	GAGDVAFVKH	STVFDNLPNP	EDRKNYELLC	
501						
	GDNTRKSVDD	YQECYLAMVP	SHAVVARTVG	GKEDVIWELL	NHAQEHFGKD	
601						
	KPDNFQLFQS	PHGKDLLFKD	SADGFLKIPS	KMDFELYLGY	EYVTALQNLR	
01						
	ESKPPDSSKD	ECMVKWCAIG	HOERTKCDRW	SGFSGGAIEC	ETAENTEECI	
01						
	AKTMKGRADA	MSLDGGYLYI	AGECGLVPVL	ARNYKTRGES	CENTPREGYL	
01					211122 2110 22	
	avavvergea	N T MANNER KENK	RECHTAVERT	A CHART PAGE T.	VSKINNCKED	
	seese end of the Day	as a severe INTO LUCK	THE OTHER PARTY OF T	AND WAR AF PROLIDE	a conta a a do fre D	~

This view is helpful when building a summary that can be sent to a customer/collaborator. Refer to <u>15 Exporting</u> Data/Reports and Printing 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 matched peptides, are clustered together. To expand and collapse the full list of proteins within each cluster click the '+' or '-' sign respectively.

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

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
Coverage Display	A graphical coverage map. Blue areas represent parts of the sequence that have been explained by the identified peptides.
PEAKS (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.
Description	The part of the protein's header information as parsed from the database. Usually it contains the name of the protein.
Marked	A multi-function checkbox. By default unchecked, but we can use this to select proteins for export or multiple sequence alignment.

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 displayed. Click any of them to show or hide a column. These settings will apply to all your reports.

# Peptides

The "Peptides" tab displays information about the peptides that match the selected protein. This list is identical to the "Peptide View" panel. Please refer to section <u>7.2.1 Peptide View</u> for more details.

Peptides Coverage	eptides Coverage Tool Box														
ID	Sequence	PEAKS(Score %)	p-value	M/Z	z	Mr(Calc)	Delta(Mass)	Error(ppm)	RT	Scan	Quality	Start	End	# Hits	R.
Spectrum 1	DNPQTHYYAVAVVK	99.0	1.4E-45	802.9082	2	1603.7993	-0.0026	1.5984	0.07	2	0.788	107	120		
Spectrum 2	TSDANINWNNLK	98.6	1.4E-45	695.3397	2	1388.6685	0.0035	2.5492	0.31	4	0.785	457	468		1
🖮 🌑 Hit 4	HSTVFDNLPNPEDR	99.0	1.4E-45	820.8843	2	1639.759	0.0049	2.9778	1.42	8	0.787	230	243	2	
Spectr	HSTVFDNLPNPEDR	97.1	1.4E-45	547.5921	3	1639.759	0.0045	2.7544	1.34	7	0.777				
Spectr	HSTVFDNLPNPEDR	99.0	1.4E-45	820.8843	2	1639.759	0.0049	2.9778	1.42	8	0.787				1

## Coverage

Beside the "Peptide" tab, you will see the coverage tab, which contains the protein sequence with the matching peptide sequences underlined in blue. The darker the blue underline, the more confident the match.

1	MRPAVRALLA CAVLGLCLAD PERTVRWCTI STHEANKCAS FRENVLRILE SGPFVSCVKK
61	TSHMDCIKAI SNNEADAVTL DGGLVYEAGL KPNNLKPVVA EFHGTKDNPQ THYYAVAVVK
121	KDTDFKLNEL RGKKSCHTGL GRSAGWNIPM AKLYKELPDP QESIQRAAAN FFSASCVPCA
181	DQSSPPKLCQ LCAGKGTDKC ACSNHEPYFG YSGAFKCLME GAGDVAFVKH STVFDNLPNP
241	EDRKNYELLC GDNTRKSVDD YQECYLAMVP SHAVVARTVG GKEDVIWELL NHAQEHFGKD
301	KPDNFQLFQS PHGKDLLFKD SADGFLKIPS KMDFELYLGY EYVTALQNLR ESKPPDSSKD
361	ECMVKWCAIG HQERTKCDRW SGFSGGAIEC ETAENTEECI AKIMKGEADA MSLDGGYLYI
421	AGKCGLVPVL AENYKTEGES CKNTPEKGYL AVAVVKTSDA NINWNNLKDK KSCHTAVDRT
481	AGWNIPMGLL YSKINNCKFD EFFSAGCAPG SPRNSSLCAL CIGSEKGTGK ECVPNSNERY
541	YGYTGAFRCL VEKGDVAFVK DQTVIQNTDG NNNEAWAKNL KKENFEVLCK DGTRKPVTDA
601	ENCHLARGPN HAVVSRKDKA TCVEKILNKQ QDDFGKSVTD CTSNFCLFQS NSKDLLFRDD

661 TKCLASIAKK TYDSYLGDDY VRAMTNLRQC STSKLLEACT FHKP

# Assigned/ UnAssigned De Novo View

The assigned de novo view displays de novo sequences that do have corresponding database matches. The assigned de novo view displays the de novo sequences that do have a corresponding database match. The two together are equivalent to the standalone de novo results.



# **Tool Box**

Click on the "Tool Box" tab:

The selected protein will be displayed at the top of this page. Click on NCBI Blast Search or NCBI Entrez Search to search for the selected protein sequence in the NCBI database or the Entrez database, respectively.

To perform a multiple alignment search mark two or more proteins in the above list of proteins, by clicking in their checkboxes (see example below).



Acc	ession	ID	Mass	Coverage Display	PEAKS(Score %) ∇	p-value	Coverage(%)	Query matched	Description	Marked
	DB Search									
	Q29443 TRFE_BOVIN	1	77753.200		98.3	8.25E-135	5.68	4	Serotransferrin - Bos taurus (Bovine).	<b>V</b>
	💭 P02769 ALBU_BOVIN		69293.550		83.8	1.87E-60	3.62		Serum albumin - Bos taurus (Bovine).	V
	P00330 ADH1_YEAST	3	36691.957		59.5	4.2E-45	2.59	1	Alcohol dehydrogenase 1 - Saccharomyces cerevis	

Then click on the "Multiple Sequence Alignment" link in the Toolbox window to generate the multiple sequence alignment 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.

Q29443 TRFE_BOVIN	mrpavrallacavlglcladpertvrwctiSthEanKcasFrENvlRIlesgpfvscvkk
P00330 ADH1_YEAST	SipEtqKgviFyEShgKL
Q29443 TRFE_BOVIN	tsHmDcikaIsnnEADaVtLDgglVyeAGLkpnNLkpvvaeFHGtkdnPqthyyavavvk
P00330 ADH1_YEAST	-eHkDipVpkpKANeLlINVkySGVchtDLhaWHGdwplP
Q29443 TRFE_BOVIN	kdtdfKLneLrGkKschtGLGrSa-GWnIpmaklykelpdpqesiqrAaanFfSaSCV
P00330 ADH1_YEAST	vKLplVgGhEgagvvvGMGeNvkGWkIgdyAgikWlNgSCM
Q29443 TRFE_BOVIN	pCadqssfpKlCQLcagkgtDKcaCsnHepyfGYSGAF-KclmegAgdvAfVkhsTvf
P00330 ADH1_YEAST	aCEyCELgNEsnCp-HadlsGYThdGSFqQyatadAvqaAhIpqgTdl
Q29443 TRFE_BOVIN	dnLpnpedrknyeLLCgdnTrksvddyqecYlAMvpSHaVvartvggkedviWelLNhAq
P00330 ADH1_YEAST	aqVapILCagiTvYkALksANlMaghWvaISgAa

# 7.2.3 COMBINE DATASETS FOR ANALYSIS

PEAKS provides a function to combine datasets for protein/peptide identification.

# Sample Level Identification

When you select a sample to perform PEAKS DB search, PEAKS will combine all datasets (files) within this sample together and generate a single result report.

# **Project Level Identification**

When you select a whole project to perform PEAKS DB search, PEAKS will combine all samples in this project together and generate a single result report.

# 7.2.4 FILTER OPTIONS

Previous users may recall the Filter Options pane. These options have been removed and re-displayed with greater prominence as part of the Assigned De Novo View and Unassigned De Novo View.



# Chapter

# 8 PTM FINDER

# 8.1 SETTING UP PTM FINDER PARAMETERS

Select a Protein ID results file to perform a PTM finder search.

# Note that you cannot execute PTM Finder on a raw file or de novo results.

Click the PTM icon on the toolbar Cr Select "PTM Finder" from the Tools menu. The "PTM Finder Options" window will appear:

The presented parameters are identical to those used when performing protein ID (Refer to section <u>7.1 Setting up Protein</u> <u>Identification Parameters</u>) 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 ALC (%) score greater than: The PTM Finder requires a good quality 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 50%.

PTM Finder	Σ
ools	PTM Finder Predefined parameters Instrument_default
	Mass Options
Data Refinement	Parent Mass Error Tolerance: 20 ppm - Precursor Mass Search Type:
Replicate Analysis	Fragment Mass Error Tolerance: 0.8 Da -
De Novo	Enzyme
prive en aut	Trypsin View Enzyme New Enzyme
PEAKS Search	РТМ
SPIDER Search	Deamidation     Set PTM
PTM Finder	F Carboxymethyl Remove Switch type
	Maximum allowed variable PTM per peptide 3
	General Options
	Preprocess this data 'on the fly' Max Missed Cleavages: 1 -
	Filter Options Filter the spectra which satisfy the following conditions for use in the PTM search:
	De novo ALC (%) score greater than: 50 % recommend 50%
	Protein ID peptide score less than: 65 % recommend 65%
	OK Cancel Help

Protein ID peptide score less than: As 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 PTM Finder will only be performed on peptides below the threshold. The recommended threshold is 65%.

# 8.2 PTM FINDER RESULTS VIEW

The results from a PTM finder search are presented in a similar format to those seen in a PEAKS Protein ID search. Please refer to section <u>7.2 Protein Identification Results</u> for more information on the PEAKS Protein ID search results.

# Chapter 0

# 9 SPIDER SEARCH

After having obtained de novo sequences for peptides that are not in the database, it is important to look for a homologous peptide in the database (Han et al., 2005). This will help you to learn more about the proteins in your sample. To search with SPIDER you must first have good quality de novo sequences.

# 9.1 SETTING UP SPIDER PARAMETERS

The configuration panel of SPIDER Search will be invoked by selecting a data file, a de novo result or a protein id result and clicking the SPIDER icon on the toolbar or choosing SPIDER Search from the Tools menu. If SPIDER search is launched on a data file, de novo sequencing will be performed automatically with selected parameters. The configuration panel is as follows:

If SPIDER search is launched from a de novo result, PEAKS will use the existing tags in the de novo results.

If SPIDER search is launched from a PEAKS Protein ID result, we do not need to select a protein database or de novo sequence. A SPIDER search performed on a Protein ID result will produce a search based the proteins on identified in the Protein ID result, whereas a SPIDER search done on a data file will create new de novo results and search then against an entire database, as specified by the user.

\land SPIDER Search	
Tools	SPIDER Search Predefined parameters default
	General Options
. Data Refinement	Query Type:      Tag Match      Homology Match     Report Top:
. Replicate Analysis	Mass Error Tolerance
. De Novo	Mass Tolerance: 0.2 Da 🛛 Leucine=Isoleucine 📝 Lysine=Glutamine
. PEAKS Search	PTM
√ SPIDER Search	Set PTM
. PTM Finder	Remove
	Database
	Database: Incbi
	Taxa: all species Set/View taxa
	De Novo Tags
	Use new de novo with predefined parameters: please select Instrument_default -
	OK Cancel Help

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

# 9.1.1 GENERAL OPTIONS

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

Tag 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. This search mode allows for the use of fixed modifications.

Homology Match: this is a more rigorous (and a more resource intensive) search mode, taking into account all types of mutations and the positional confidence scores. This search mode also creates reconstructed peptides that use information from both the de novo sequences and the database sequence in order to characterize the real sequence. This search mode allows for the use of both fixed and variable modifications.

You can choose how many of the best homologous peptides should be displayed after searching by changing the number in the "Report Top" box.

# 9.1.2 MASS ERROR TOLERANCE

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

Choose if you would like PEAKS to consider Leucine equal to Isoleucine without a penalty in the score and also whether Lysine should be equal to Glutamine without penalty by selecting the appropriate checkboxes.

# 9.1.3 PTM

Set PTM: Clicking this button will bring up the "PTM Options" list, which informs PEAKS what types of posttranslational modifications to include in its analysis. To view additional modifications, select the "Show unimod" box. If the desired PTM does not appear on the list or is different than what is listed, you can select the "New…" button and the "PTM Editing" window will open. Fill in the information pertaining to your particular PTM. For a more in depth explanation of creating a new PTM, refer to section <u>7.1.3 PTM Options</u>.

To select a PTM as Fixed or Variable, click the desired PTM to highlight it and then click the button corresponding to its preferred designation, "Fixed Modification" or "Variable Modification". If you incorrectly place a PTM in one of these sections, simply reselect the PTM, and drag it out to have it removed from the list of selected modifications, or click "Switch Type" to have it set as the other modification type. For example, using the Switch Type button, a PTM currently listed as fixed, will automatically be removed from the fixed modification list and enter the variable modification list, or vice versa.

# Note that in previous versions of PEAKS, only fixed PTMs were allowed; however, PEAKS now allows variable PTMs, while executing a homology 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).

# 9.1.4 FILTER

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 (ALC %) score greater than: The SPIDER search requires a good quality 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 50%.

Protein ID peptide score less than: As there is no need to run SPIDER on peptides that have already been found to have a good match during PEAKS Protein ID, it is helpful to enter a peptide score threshold so that SPIDER will only search through peptides below the threshold. The recommended threshold is 65%.

# 9.1.5 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 result file. Selecting a database provides sequence variations for SPIDER to search against de novo sequences.

Database to search: From the drop-down list select 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 where you have the option of deleting databases which you have created. For more information on setting up new databases refer to section <u>16.2.3 Database Configuration</u>.

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/View 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.

After setting up the desired parameters, we can save them for future use. Click the "Save Parameters" button, and define name for these preferences for future use/reference, when prompted. Any parameters that you save will be available in the drop-down list at the top of the window. To examine the contents, select a saved parameters file and parameter boxes will be populated.

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

# 9.2 SPIDER RESULTS VIEW

SPIDER will search the database for homologous peptides and at the same time attempt to consolidate these into protein hits. 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. Refer to section <u>7.2.1 Peptide View</u> for more details. Click on the "Peptide details" tab to see the SPIDER matches shown in red. Note that only SPIDER homology search results will display a reconstructed or "real" sequence.



Letters on a green background 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. [Square brackets] indicate an equal mass substitution, common non-critical de novo errors. While <Angle brackets> indicate an equal mass substitution and a mutation.

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

Clicking on "Protein View" will yield a similar display as was seen for PEAKS Protein ID (refer to section <u>7.2.2</u> <u>Protein View</u>). In the "Coverage Display" column, red region indicate areas of homology and potential mutation identified by SPIDER. Blue regions indicate areas of homology from the protein ID search, and purple indicates that the spectrum was identified by both SPIDER and protein ID.

Accession	ID	Mass	Coverage Display	PEAKS(Score %) ∇	p-value	Coverage(%)	Query matched	Description	Ę
🖃 🌑 DB Search									*
Q29443 TRFE_BOVIN	1	77753		98.3	8.25E	8.52	7	Serotransferrin - Bos taurus (Bovine).	
P02769 ALBU_BOVIN	2	69293		83.8	1.87E-60	5.27	5	Serum albumin - Bos taurus (Bovine).	
P00330 ADH1_YEAST	3	36691	1	59.5	4.2E-45	2.59	2	Alcohol dehydrogenase 1 - Saccharomyces cere	

Click on the "Coverage" tab. The highlighted colors are the same as those shown in the "Display" column above.

Peptides Coverage Tool Box
1 MRPAVRALLA CAVLGLCLAD PERTVRWCTI STHEANKCAS FRENVLRILE SGPFVSCVKK
61 TSHMDCIKAI SNNEADAVTL DGGLVYEAGL KPNNLKPVVA EFHGTKDNPQ THYYAVAVVK
121 KDTDFKLNEL RGKKSCHTGL GRSAGWNIPM AKLYKELPDP QESIQRAAAN FFSASCVPCA
181 DQSSFPKLCQ LCAGKGTDKC ACSNHEPYFG YSGAFKCLME GAGDVAFVKH STVFDNLPNP
241 EDRKNYELLC GDNTRKSVDD YQECYLAMVP SHAVVARTVG GKEDVIWELL NHAQEHFGKD
301 KPDNFQLFQS PHGKDLLFKD SADGFLKIPS KMDFELYLGY EYVTALQNLR ESKPPDSSKD
361 ECMVKWCAIG HQERTKCDRW SGFSGGAIEC ETAENTEECI AKIMKGEADA MSLDGGYLYI
421 AGKCGLVPVL AENYKTEGES CKNTPEKGYL AVAVVK <mark>TSDA NINWNNLK</mark> DK KSCHTAVDRT
481 AGWNIPMGLL YSKINNCKFD EFFSAGCAPG SPRNSSLCAL CIGSEKGTGK ECVPNSNERY
541 YGYTGAFRCL VEKGDVAFVK DQTVIQNTDG NNNEAWAKNL KKENFEVLCK DGTRKPVTDA
601 ENCHLARGPN HAVVSRKDKA TCVEKILNKQ QDDFGKSVTD CTSNFCLFQS NSKDLLFRDD
661 TKCLASIAKK TYDSYLGDDY VRAMTNLRQC STSKLLEACT FHKP
Mutated Peptide Exact Match

# Chapter

# 10 INCHORUS (MULTI-ENGINE PROTEIN ID)

The inChorus protein identification tool can call upon several search engines (PEAKS Protein ID, X!Tandem, OMSSA, Mascot and Sequest) for protein identification and will then compare and summarize the results from the different search engines in one unified report.

Please note that if you wish to launch either Mascot or Sequest for an inChorus search, these engines are not internally provided with PEAKS and may need to be purchased from their respective vendors; however, licenses of these engines are not required if the result files have already been obtained. To set up your search engine preferences, refer to section <u>16.1.3 Search Engine Preferences</u>.

# 10.1 SETTING UP INCHORUS PARAMETERS

Select the project, sample, or data file node to be run through inChorus.

Click the "inChorus Search" icon on the toolbar Or Select "inChorus Search" from the Tools menu.

The "inChorus Options" window will appear:

First select each of the protein identification tools that you would like to use by checking the respective checkboxes. Search parameters for each program can be set by selecting the name of the search engine.

The option screens for each of the search engines available to inChorus are designed to work in the same way as options screens from 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 to section <u>7.1 Setting up</u> Protein Identification Parameters.

inChorus Search	
Tools	PEAKS Protein ID
PEAKS Protein ID	Database Search Predefined parameters Instrument_default  Save as
SPIDER	Error Tolerance
X!Tandem	Parent ion: 0.01 Da 🗸 using monoisotopic mass 🗸 Fragment ion: 0.5 Da
OM55A	Enzyme Trypsin View Enzyme New Enzyme
Mascot	Maximum missed cleavages per peptide:
Sequest	РТМ
Import Result	Set PTM Remove Switch type
	Database
	Select database Database: Sample DB      View Edit     Paste sequence Taxa: all species     Set/View taxa
	General Options Validation with reverse database as decoy
	OK Cancel Help

# **10.1.1 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 in the results file is consistent with the one in PEAKS. PEAKS uses original data information to compute the inChorus score.

When you run an 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

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 drop-down 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 <u>16.2.3</u> <u>Database Configuration</u> to configure your databases. To specify which taxa you would like to search, click on the "Set Taxa" button.

Tools	Import Result Predefined parameters Save as
PEAKS Protein ID	Database Define a common database for importing search result.
SPIDER	Database to search Sample DB 🔹
X!Tandem	Taxonomy selections all species
OMSSA	Set Taxa
Mascot	
Sequest	Import Search Result Import XTandem .xml file Browsee
🖉 Import Result	Import Omssa .omx/.xml file Browse
	Import Mascot .det file Browse
	Import Sequest .srf file Browse
	OK Cancel He

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 the 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 result node 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:

# 

# 10.2.1 DE NOVO, PEPTIDE AND PROTEIN VIEWS

Each result node for the 3rd party search engines looks very similar to the PEAKS Protein ID result node (Protein Identification Results) 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 third party search engines.

The inChorus search report also looks very similar to PEAKS Protein ID results file. 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.



# 11 PEAKS<sup>®</sup> QUANTIFICATION MODULE

Many approaches for quantifying proteins using mass spectrometry have been described in the literature (ref). 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 (TMTs).
- Label free: This quantification type is based on the relative intensities of extracted ion chromatograms (XICs) for precursors in multiple data sets aligned using mass and elution time. Please note PEAKS requires the survey scan to be run in profile mode.

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



# 11.1 SETTING UP PEAKS Q PARAMETERS

# 11.1.1 PARAMETERS FOR LABELED QUANTIFICATION

1) In the "Project View Frame", select a  $\checkmark$  PEAKS Search result file.

2) Click the PEAKS quantification  $\bigcirc$  toolbar icon. Or

Select "Quantification" from the "Tools" menu.

The following window will open, displaying the labeled quantification parameters:

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

	ITRAQ			default 👻 Save	16
	Basic Options				
<ul> <li>Label at MS/MS level eg. iTRAQ</li> </ul>	Mass Error Tolerance:	0.1 Da 💌	Upper Bound o Peptide Score 1	f Precursor Charge: 4	
D Label at MS level eg. 3CAT	Label Options				
	Label Name	Berneter Ivo (Da)		shellon Efficiency (%)	
C Label Pree	51	Papartar 201(Day	114.1	1.0	
	52		115.1	1.0	
	53		116.1	1.0	
	54		117.1	1.0	
		Add Label	Delete Label		
				OK Canoel	
					_
Quantification					
antifications	N'AT			daladi - Same	
	1.01				
	Basis Onlines				
	base Options	0.1	ike a firmed a	And the second se	
D Label at MS/MS level	Mass Error Tolerance:	0.1 Da •	Upper bound o	Precursor Charge: 4	
	Retention Time Range:	1.0 min. •	Peptide Score 1	Threshold: 50 %	
Label at MS level					
eg. ICAT	Label Options				
	Label Name	udded Mass	Residues	Labeling Efficiency (%)	
C. Label From	51	6.0	K	1.0	
🗇 Label Free	P			1.0	
🗇 Label Free	52	0.0	~	1.0	
🖰 Label Pree	52	0.0		1.0	
🖰 Label Pree	8	0.0		1.0	
🖰 Label Pree	52	Add Label	Delete Label	3.0	
D Label Pree	2	Add Label	Delete Label	1.0	
🗇 Label Free	52	Add Label	Delete Label	1.0	
🛛 Label Free	52	AddLabel	Delete Label	1.0	
🛛 Label Free	52	AddLabel	Delete Label	3.0	

Once a quantification type is selected, many quantification parameter options are included.

These 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 be present in different charge states. Upper bound of precursor charge defines the maximum charge of peptides which are used for counting quantity.
- Peptide Score Threshold: Only those identified peptides with score above this threshold will be used in quantification.
- Retention Time Range: The retention time range is for pairing up features.

# Labeling Tools

- 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 MS level e.g. SILAC: It is for quantification based on the relative intensities of extracted ion chromatograms (XICs) for precursors within a single data set.
- MS Label Options
- Label Name: It is for specifying label name which cannot be left empty
- Reporter Ion: It is for specifying mass of reporter ion.
- Labeling Efficiency: It is for specifying efficiency of chemical reaction.
- Add Label: Click on this button will add a default label.
- Delete Label: Select a label and Press this button, the selected label will be deleted.

# MS/MS Label Options

- Label Name: It is for specifying label name which cannot be left empty
- Added Mass: The modified mass of a residue.
- Residues: The residues to be modified given in single codes of amino acids, i.e. A or AK
- Labeling Efficiency: It is for specifying efficiency of chemical reaction.
- Add Label: Click on this button will add a default label.
- Delete Label: Select a label and Press this button, the selected label will be deleted.

# 11.1.2 PARAMETERS FOR LABEL FREE QUANTIFICATION

 In the "Project View Frame", select any node of the project you want to do label free quantification. The project should have at least one PEAKS Search result.
 Click the PEAKS quantification toolbar icon

Q. Or Select "Quantification" from the "Tools" menu.

The following window will open, displaying the label free quantification parameters:

## **Basic Options**

- Mass Error Tolerance: The mass error tolerance is for pairing up features.
- Retention Time Range: The retention time range is for pairing up features. Only the feature retention time within this range may pairing up.
- Peptide Score: Only those identified peptides with score within the given range will be used in quantification.
- Protein Score: Only those identified proteins with score within the given range will be quantified
- Upper Bound of Precursor Charge: The peptide may be present in different charge states. Upper bound of
  precursor charge defines the maximum charge of peptides which are used for counting quantity.
- Do normalization: If this option is selected, normalization to protein ratio which based on total ion intensity will be done automatically.

## Label-Free Parameter Table

- Project Name: Gives the name of the projects selected to do quantification
- Sample Name: Gives the names of samples in the project
- Fraction Number: Gives the number of the fraction in the sample
- File Name: Gives the name of the data file
- Protein ID: Gives the name of the PEAKS node will be used in quantification
- Add to Quantification: Check/uncheck to add the sample to the quantification. There must be at least two
  samples in label free quantification and the number of fraction within each sample must be the same.
- Up/Down: If the order of fractions in the sample is not consistent with that in the experiment, these two buttons can be used to adjust the order.

# **11.1.3 SAVING PARAMETERS**

Clicking the "Save As" button at the top right allows the user to save parameters for ease of use when regularly performing quantification with the same parameters. All the parameters in quantification will be saved except the project table in label free quantification which will change from one project to another.

# 11.2 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.

# 11.2.1 SETTING UP A 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:\PeaksStudio 5.2\Data).

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

FASTA forma	at database:	UniProtKB/Swiss-Prot	•	Validate Database
Basic Option	s			
Database r	name:	Sample DB	Validated	
Path:	C:\PEAKS_Stu	udio_5.2\Data\SampleDB.fasta	Browse or	Download Database
🔲 EST da	tabase			

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

# 11.2.2 CREATING A PROJECT

Click on the "Create new project" icon for select "New project" from the "File" menu. Give a name to your project, for example "iTRAQ Data". Click Add data files. Locate "iTRAQSample.mzxml" from C:\PeaksStudio5.2\Data.

With the file highlighted, select, Quad-TOF from the "Instrument Type" drop-down list.

Click "OK". Wait until the file has

roject Name	iTRAQ Data	
roject Location	C:\PEAKS_Studio_5.2\.\derb	yServer\serverDB Browse
Data Files		Data Details
Data Files ITRAQ Data → Sample 1 → Add data files		Data File Name: C:\PEAKS_Studio_5.2\Data\;TRAQSample.mzXML Instrument Vendor: All Instruments • Instrument Type Quad-TOF • Additionally apply same instrument config to whole project whole sample
Delete Up	Down Add Sample	Sort by Replicate OK Cancel

loaded.

Double click on the iTRAQ sample file.

Your "Main Processing Screen" should look something like this:

For ease of setup, this walkthrough will use the workflow tool in order to set all of the parameters at once.



# 11.2.3 SETTING UP THE WORKFLOW PARAMETERS

Click on the workflow button "W" and select "Quantification". The following window will appear:

Click "Select Data". Select the iTRAQSample.mzXML file and then click "Add to Right". The samples should now appear in the "Selected Data" panel.

Click "OK". The workflow window should now display "finished" beside the "Selected Data" button then click the "Refine data" button.

Enter the following parameters:

- Correct Precursor Mass
- Correct Precursor Charge State:
  - Min 1, Max 4
- Filter Scan:
  - Quality value greater than 0.65
- Data Preprocess: Yes

Work Flow Configuration [3]
M5/M5 Data Refinement
Merge Scans
Retention time window (for raw files only): min
Precursor m/z error tolerance:
Correct Precursor Mass
Correct Precursor Charge States
Min charge: 1 🚖 Max charge: 4 🚖
✓ Filter Scans
Only keep scans satisfying:
Precursor mass between and Da
Retention time between and min
Quality value greater than 0.65 (suggest 0.65)
Data Preprocess (peak centroiding, charge deconvolution, and deisotope)
💿 no, already done 🛛 🛞 yes 👘 no
OK Cancel



Click "OK". Then click the "de novo" button. Enter the following parameters:

- Parent ion: 0.1 Da
- Fragment ion: 0.1 Da
- Enzyme: Trypsin
- PTM:
  - AB 4plex (Y) and Oxidation M as variable.
  - AB 4plex (K), (N) and Carbamidomethylation as fixed.
- Min allowed variable PTM: 3

Click "OK" and then click the "PEAKS Search" button.

Enter the following parameters:

- Parent ion: 0.1 Da
- Fragment ion: 0.1 Da
- Enzyme: Semi Trypsin
- PTM:
  - AB 4plex (Y) and Oxidation M as variable.
  - AB 4plex (K), (N) and Carbamidomethylation as fixed.
- Min allowed variable PTM: 3

Click the "Quantification" button. Select "Label at the MS/MS level" from the left hand panel and enter the following parameters:

- Mass Error Tolerance: 0.1 Da
- Upper Bound of Precursor Charge: 4

To add information into the

Peptide Score Threshold: 60

Note:

Juantifications	ITRAQ		default 👻 Save	e as
. Label at MS/MS level	Basic Options Mass Error Tolerance:	0.1 Da 👻 Upper Bou	nd of Precursor Charge: 4	÷
eg. iTRAQ		Peptide Sc	ore Threshold:	60 %
Label at MS level eg. ICAT	Label Options			
Label Free	Label Name	Reporter Ion (Da)	Labelling Efficiency (%)	
	51	114.1:	12	1.0
	52	117.11	5	1.0
		Add Label Delete Label	]	

"Label Options" panel, click the "Add Label" button to add a new sample.

Add labels S1: 114.112 & S2: 117.115



Predefined parameters ITRAQ sample (db)

sotopic mass 👻

Fragment ion: 0.1

▼ Save as...

Set PTM... Remove

Switch type

▼ New... Edit...

Set/View taxa...

OK Cancel

Da

View Enzyme New Enzyme...

Database Search

Error Tolerance

Da - using m

Maximum missed cleavages per peptide: 1 -

Appled Biosystems ITRAQ(TM) 4plex (Y)
 Oxidation M
 Appled Biosystems ITRAQ(TM) 4plex (k)
 F Appled Biosystems ITRAQ(TM) 4plex (k)
 Carbamidomethylation

Naximum allowed variable PTM per peptide 3 🚖

Taxa: all species

Perform on-the-fly preprocessing (peak centroiding, charge deconvolution, and deisotope)

(If you want to do protein search with different De Novo parameter, please use WorkFlow or select a De Novo node to start)

Select database Database: Sample DB

Validation with reverse database as decoy

Parent ion: 0.1

Enzyme

Trypsin

Database

Paste sequence

General Options

PTM

Click "OK" and then click "Start" in the workflow configuration panel. The following window will open:

Click "Start Jobs".



# 11.2.4 ITRAQ RESULTS

Once completed, the protein quantification result will be displayed in the Quantification node a. Double click on this node and the "Peptide View" tab will appear by default. The quantification results are listed as a "Ratio of 117.115:114.112":

Click on the "Peptide Details" tab to see a simple alignment between the original de novo sequence, the peptide found in the database and the reconstructed sequence. At the bottom of the "Peptide Details" panel you will see where the selected peptide matches the protein highlighted in blue.

PEAKS provides the 3D View of each peptide feature for visual validation. Click on the 3D View tab. The panel along the bottom allows you to focus 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.





Select the "Protein View" tab. The quantification results are listed as a "Ratio of 117.115:114.112" and as "Standard Deviation of 117.115:114.112". They are highlighted in the blue box below. For example the relative protein ratio for the top ranked protein (Beta-galactosidase) is 4.26 with a standard derivation of 0.46.

	ouant 🛃	ITATION 4 ×														
View	Accession	I	Mass	Coverage	Display	PEAKS(Sco	ore %)	p-value	Covera	ge(%) Des	ription	Ratio	117.115	:114.112	SD 117.115:114.112	R.
E.	🖃 🌑 DB Search															*
Prol	🖳 💭 P00722 B	GAL_ECOLI	116351			95.	.2 f	5.19E	4.3	3 Beta	galactosidase		4.26		0.46	-
Peptide Vi	Peptides Coverage	e Tool Box Sequence			PEAKS(	Score %)	p-value	M/Z	Z	Mr(Calc)	Error(ppm)	RT	Scan	Quality	117.115:114.112	Ę
iew	Spectrum 1	A(+144.10)F	LDNDIGVSEAT	rr.		99.0	1.4E-45	801.353	3 2	1600.8176	77.7861	0.04	2	0.79	4.59	
0	Spectrum 5	D(+144.10)	ENPGVTQLNF	ર		91.8	1.4E-45	786.898	4 2	1571.7815	0.5436	0.42	8	0.79	3.94	
2	Spectrum 7	M(+144.10)9	GIFR		-	47.3	1.26E-6	427.727	2	853.46027	24.3872	0.59	11	0.777	5.07	
I De	Spectrum 8	V(+144.10)D	EDQPFPAVPK	(+144.10)		48.7	8.88E-16	815.418	1 2	1628.8652	26.755	0.76	13	0.791	4.55	

# 11.3 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.

# 11.3.1 Setting up a 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 5.2\Data).

Click on the configuration toolbar icon select "Configuration" from the "Tools" menu. Select "Database" from the left hand side of the window. Under "Database Details" enter the following information:

You do not need	FASTA format database:	UniProtKB/Swiss-Prot	•	Validate Database
to change any of	Basic Options			
the other	Database name:	Sample DB	Validated	
information				
listed. Click the	Path: C:\PEAKS_St	udio_5.2\Data\SampleDB.fasta	Browse or	Download Database
"Add/Update"	📰 EST database			
button and then				
click "OK".				

# 11.3.2 CREATING A PROJECT

Click on the "Create new project" icon or select "New project" from the "File" menu. Give a name to your project, for example "SILAC Sample". Click Add data files. Locate "SILACSample.mzxml" from C:\PeaksStudio5.2\Data.

With the file highlighted, select, FT-trap from the "Instrument Type" drop-down list. Click "OK".

🔨 New Project			83					
Project Name	SILAC							
Project Location	C:\PEAKS_Studio_5.2\.\derbyServ	rer\serverDB	Browse					
Data Files	16 mt298	Data Details Data File Name: CIPEARS_Stude_5.2l/backStLACSen Instrument Vendor: All Instruments Instrument Type FI-trap Additionally apply some instrument config whole project whole sample	ple.mc/ML					
Delete Up	Down Add Sample	Sort by Replicate OK	Cancel					

Double click on the SILAC sample file. Your "Main Processing Screen" should look something like this:

For ease of setup, this walkthrough will use the workflow tool in order to set all of the parameters at once. You will need to wait until the file has loaded (i.e. the file node looks like this: •).



# 11.3.3 SETTING UP THE WORKFLOW PARAMETERS

Click on the workflow button ". The following window will appear.

Click "Select Data". Select the SILACSample.mzXML file and then click "Add to Right". The samples should now appear in the "Selected Data" panel. Click "OK".

Check the 2nd box and then click the "Refine data" button. Enter the following parameters:

- Correct Precursor Mass
- Correct Precursor Charge State:
  - o Min 1, Max 3
- Filter Scan:
  - Quality value greater than 0.65
- Data Preprocess: Yes

# Click "OK".

Check the 3rd box and then click the "de novo" button. Enter the following parameters:

- Parent ion: 0.1 Da
- Fragment ion: 0.8 Da
- Enzyme: Trypsin
- **PTM**:
  - K6 and Oxidation M as variable.
  - Carbamidomethylation as fixed.
- Min allowed variable PTM: 3

## Click "OK".

Check the 4th box and then click the "PEAKS Search" button. Enter the following parameters:

- Parent ion: 0.1 Da
- Fragment ion: 0.8 Da
- Enzyme: Trypsin
- PTM:
  - o K6 and Oxidation M as variable.
  - o Carbamidomethylation as fixed.
- Min allowed variable PTM: 3

Click "OK".

nojno Data kerinenent		
Merge Scans		
Retention time window (for raw	iles only): min	
Precursor m/z error tolerance:	🗇 Da 💮 ppm	
Correct Precursor Mass		
Correct Precursor Char	e States	
Min charge: 1 🚔 Max o	narge: 3 🌩	
Filter Scans Only keep scans satisfying:		
Precursor mass between	and Da	
Retention time between	and min	
🔽 Quality value greater than	0.65 (suggest 0.65)	
Data Dronrocoss (neak centr	ding, charge deconvolution, and dejectorie)	
o oo ahaadu daraa 🔊 a		
Tid, already done	5 010	




Check the 5th box and then click the "Quantification" button. Select "Label at the MS level" from the left hand panel and enter the following parameters:

- Mass Error Tolerance: 0.1 Da
- Upper Bound of Precursor Charge: 4
- Retention Time Range: 1.0 min
- Peptide Score Threshold: 60

Note: To add information into the "Label Options" panel, click the "Add Label" button to add a new sample.

Label: Light, with added mass 0.0 on residue K.

Label: Heavy, with added mass 6.0 on residue K

Click "OK" and then click "Start" in the workflow configuration panel. The following window will open:

Click "Start Jobs".

#### 11.3.4 SILAC RESULTS

Once completed, the protein quantification result will be displayed in the PEAKS Protein ID node "<sup>(1)</sup>". Double click on this node and the "Peptide View" results will appear by default:

Clicking on the "Peptide Details" tab will display selected peptide matches in the protein highlighted in blue.

PEAKS provides the 3D View of each peptide feature for visual validation. Select the "3D View" tab. The panel along the bottom allows you to focus 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.



uantifications	ICAT			def auit.	<ul> <li>Save as</li> </ul>
	Basic Options				
Label at MS/MS level	Mass Error Tolerance:	0.1 Da •	<ul> <li>Upper Bound of Pr</li> </ul>	ecursor Charge: 4 🕀	
eg. iTRAQ	Detection Time Banner	1.0 [nin	Peoplide Score The	-dv86 60	w.
	receiver the range.	1.0			~
Label at MS level eg. ICAT	Label Options				
Label Free	Label Name	Added Mass	Residues	Labeling Efficiency (%)	
1000011100	Light		D.OK		1.0
	Heavy		1.0 K		1.0

Select the "Protein View" tab. The SILAC quantification results are listed as a "Ratio Heavy: Light" and "Standard Deviation Heavy: Light".

Note that the top protein result is Human Filamin A, with a score of 98.89%. The ratio of Heavy: Light is highlighted for each protein 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 0.13.

A ratio of -1 means that while a reporter ion was found for the selected peptide, a ratio could not be calculated for the protein as the peptide did not meet the required score criteria (in this case we specified that the peptide score had

to be greater than 0.6). A blank box in the ratio column means that a reporter ion for the selected peptide was not found. There are four peptide features identified and calculated for quantification of Human Filamin-A. Two other peptides (Spectrum 35 and 44) were used to identify the protein but were not identified as features.

				🛃 QUANT	TITATION 4	x							
View	Accession	ID	Mass	Coverage Display	PEAKS	(Score %)	p-value	Query mat	thed R	atio Heavy:L	ight S	D Heavy:Light	<b>₽</b>
ein	🖃 🌑 DB Search												-
P2	- 💭 P21333 FLNA_HUMAN		280736.560	1			1.06E-9	6 6					
ŝ	075369 FLNB_HUMAN	2	278192.700			61.5	2.51E-1	.3 3		-1		0	
e vi	P04264 K2C1_HUMAN	3	66017.650			34.7	4.47E-1	.0 3					=
b)	Q8TE82 S3TC1_HUMAN	11	146928.890			7.6	3.84E-	6 2					
a s	Q8N6N7 ACBD7_HUMAN	5	9790.278			15.6	1.18E-	2 1		-1		0	
Viev	P46940 IQGA1_HUMAN	6	189250.720			12.9	6.38E-	3 1		-1		0	
0,0	Q96EP1 CHFR_HUMAN	7	73386.586			12.1	4.65E-	3 1					
ž	P13497 BMP1_HUMAN	8	111248.770			11.8	1.07E-	5 1		-1		0	
ę	Q9UJX2 CDC23_HUMAN	9	68285.610	1		9.7	2.1E-2	2 1		-1		0	-
Assigne	Peptides Coverage Tool Box												
Tew P	ID Sequence			PEAKS(Score %)	p-value	M/Z	Mr(Calc)	Delta(Mass)	Error(ppn	n) Scan	Quality	Heavy:Light	R\$
8		(+6.02	)	99.0	4.1E-9	648.8403	1295.6604	-0.0057	4.4281	L 27	0.785	1.08	-
S.	Spectrum 13 DVDIIDHHDNTY	TVK(+6	.02)	66.7	7.95E-6	895.9397	1789.8579	-0.0070	3.8874	4 28	0.789	1.27	
IDe		)PTHF1	'VNAK(+6.02)	99.0	2.22E-16	855.9888	1709.9502	-0.0129	7.5671	1 38	0.789	1.31	
Dec		TVK		58.0	1.41E-7	595.6237	1783.8376	-0.0115	6.4325	5 71	0.787	1.17	
assig		VQDR		99.0	1.4E-45	882.4396	1762.8486	-0.0161	9.1404	¥ 87	0.789		1
ŝ		R		99.0	1.11E-16	1312.6951	1311.6782	-0.0095	7.259	102	0.813		1

Peptides Coverage	• Tool Box									
ID	Sequence	PEAKS(Score %)	p-value	M/Z	Error(ppm)	RT	Scan	Quality	Heavy:Light	<b>₽</b>
	YGGQPVPNFPSK(+6.02)	99.0	4.1E-9	648.8403	4.4281	0.28	27	0.785	1.08	
O Spectrum 13	DVDIIDHHDNTYTVK(+6.02)	66.7	7.95E-6	895.9397	3.8874	0.29	28	0.789	1.27	
O Spectrum 19	TGVELGK(+6.02)PTHFTVNAK(+6.02)	99.0	2.22E-16	855.9888	7.5671	0.39	38	0.789	1.31	
O Spectrum 27	DVDIIDHHDNTYTVK	58.0	1.41E-7	595.6237	6.4325	0.88	71	0.787	1.17	
O Spectrum 35	VANPSGNLTETYVQDR	99.0	1.4E-45	882.4396	9.1404	1.07	87	0.789		
Spectrum 44	VEPGLGADNSVVR	99.0	1.11E-16	1312.6951	7.259	1.22	102	0.813		

#### 11.4 LABEL FREE QUANTIFICATION

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. These unchanging analytes form the basis for identifying the non-changing concentrations, making spiking unnecessary.

#### Note: PEAKS requires the survey scan to be run in profile mode.

Below are instructions to take you through a label free dataset. Please be aware that the data files are fairly large and thus the computing time will be increased compared to the other walkthrough datasets explained in this manual.

#### 11.4.1 SETTING UP A 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 5.2\Data).

Click on the configuration toolbar icon select "Configuration" from the "Tools" menu. Select "Database" from the left hand side of the window. Under "Database Details" enter the following information:

FASTA forma	t database:	UniProtKB/Swiss-Prot	•	Validate Database
-Basic Options				
Database n	ame:	Sample DB	Validated	
Path:	C:\PEAKS_Stu	udio_5.2\Data\SampleDB.fasta	Browse or I	Download Database
📃 EST dal	tabase			

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

#### 11.4.2 CREATING A PROJECT

Click on the "Create new project" icon 🖸 or select "New project" from the "File" menu. Give a name to your project, for example "Label free walkthrough". Click Add data files. Locate LFQSample1.mzXML. Then click "Add Sample" and Sample 2 will appear. Click "Add data files" to add "LFQSample2.mzXML" to Sample 2.

# Note: For label free quantification to function in PEAKS, you need to have at least 1 sample with at least 1 file/fraction in each sample.

With one of the raw files highlighted, select, FT-trap from the "Instrument Type" drop-down. Then click "whole project" to apply these parameters to the rest of the project.

Mork Flow Configuration		82
Work How Configuration     Work How Configuration     Work Source (CR), and (CR),	Selected Data. COPERS, Studio, 5-27, Jörby/Server/Jerver/El(Label Peer Sangle 	8
All Samples All Practions Add to Right	Remove	ā
	OK Cancel	

Click "Ok".

For ease of setup, this walkthrough will use the workflow tool in order to set all of the parameters at once. You will need to wait until the files have loaded (i.e. the file node looks like this:  $\clubsuit$ ).

#### 11.4.3 SETTING UP THE WORKFLOW PARAMETERS

Click on the workflow button "W". The following window will appear:

Click "Select Data". The following window will open:

Click "All Samples" and then "Add to Right" or select the project node in the left tree and add to right. In work flow, label free quantification can only be done at the project level or at the sample level. Click "OK".

Then click the "Refine data" button. Enter the following parameters:

- Correct Precursor Mass
- Correct Precursor Charge State:
  - Min 1, Max 4
- Filter Scan:
  - Quality value greater than 0.65
- Data Preprocess: Yes

Work Flow Configuration
M5/M5 Data Refinement
Merge Scans
Retention time window (for raw files only): min
Precursor m/z error tolerance:
Correct Precursor Mass
Correct Precursor Charge States
Min charge: 1 🐳 Max charge: 4 🛬
✓ Filter Scans
Only keep scans satisfying:
Precursor mass between and Da
Retention time between and min
Quality value greater than 0.65 (suggest 0.65)
Data Preprocess (peak centroiding, charge deconvolution, and deisotope) <ul> <li>no, already done</li> <li>yes</li> <li>no</li> </ul>
OK Cancel

Click "OK".

Click the "De novo" button. Enter the following parameters:

- Parent ion: 0.1 Da
- Fragment ion: 0.8 Da
- Enzyme: Trypsin
- PTM:
  - Oxidation M and Phosphorylation-STY as variable
  - Carbamidomethylation as fixed.
- Min allowed variable PTM: 3

#### Click "OK".

Click the "PEAKS Search" button. Enter the following parameters:

- Parent ion: 0.1 Da
- Fragment ion: 0.8 Da
- Enzyme: Trypsin
- PTM:
  - Oxidation M and Phosphorylation-STY as variable
  - o Carbamidomethylation as fixed.
- Min allowed variable PTM: 3
- Click OK.

Next click the "Quantification" button. Select "Label Free" from the left hand panel and enter the following parameters:

- Mass Error Tolerance of 0.2 Da
- Peptide Score: 50 100
- Retention Time Range: 4.0 min
- Protein Score; 50-100
- Upper Bound of Precursor Charge: 4
- Do Normailization: Check

Automatically the parameter table will be configured for both samples and fraction numbers.



Error Tolerar Parent ion: 0.1 Enzyme Trypsin Maximum misser PTM Oxidation N	nce 1 Da •] d cleavages per pep	using monoisotopic m	ass 💌					
Parent ion: 0.1 Enzyme Trypsin Maximum misser PTM Oxidation N	1 Da •	using monoisotopic m	ass 💌					
Enzyme Trypsin Maximum missen PTM	d cleavages per pep			Fragme	nt ion: 0.	8 Da		
Trypsin Maximum missen PTM Oxidation N	d cleavages per pep							
Maximum misses PTM Oxidation N	id cleavages per pep			-	View	Enzyme	New E	nzyme
PTM		tide: 1 ≑						
Oxidation N								
	M						_	Set PTM
V Phosphoryl	lation-STY							Jerris
F Carbamidor	methylation							Remove
								Switch typ
Maximum allowe	ed variable PTM per	peptide 3 💠						
Database								
Select datab	base Database:	Sample D6				-	New	Edit
Paste seque	ence Taxa;	all species					Set/vie	wtaxa
General Opti Validation w Perform on (If you want to Row Configuration	ions with reverse databas withe-fly preprocessi do protein search w	se as decoy ng (peak centroiding, c ith different De Novo j	charge decor parameter, p	wolution, and d	eisotope) Flow or se	lect a De No	wo node	to start)
General Opti Validation + Perform on (If you want to Now Configuration ifications	ions with reverse databas withe-fly preprocessi i do protein search w Label Pree Sample Label Free	se as decoy ng (peak centroiding, c ith different De Novo j	charge decor parameter, p	wolution, and d please use Work	eisotope) Flow or st	lect a De No	wo node	to start)
General Opti Validation v Perform on (If you want to Flow Configuration utications	ions with reverse databas withe-fly preprocessi do protein search w Label Firee Sample Label Firee	se as decoy ng (peak centroiding, c with different De Novo j	charge decor parameter, p	wolution, and d please use Work defau	eisotope) Flow ar se it	lect a De No	wo node	to start)
General Opti Validation v Perform on (If you want to Flow Configuration Iffications el at MSJMS level (TRAQ	ions with reverse databa, -the-fly preprocess a do protein search w Label Pree Sample Label Free Basic Options More free Tripe Tripe	se as decoy ng (peak centroiding, c with different De Novo p	charge decor	wolution, and d please use Work defau	eisotope) Flow ar se k	lect a De No	wo node	to start)
General Opti Validation » Perform on (If you want to Rew Configuration flications el at H5(M5) level (TRAQ el at H5(M5) level (TRAQ	Ions with reverse databa with efly preprocessi do protein search w Label Free Sample Label Free Basic Options Mass Error Talena Rotening Options	se as decoy ng (pesk.centroiding, c ith different De Novo p en 0.2	charge decor parameter, r	volution, and d blease use Work defau Peptide Score:	eisotope) Flow or se k .	lect a De No	100 %	to start)
General Opti Validation v Perform on (If you want to Row Configuration fileations el at P5(P5) level mp/q el at P5(Ivel IoAT	Ions with reverse databa -the-fly preprocessi to do protein search w Label Free Sample Label Free Basic Options Mass Ever Toleran Retention Time Ra Upper Board Of P	se as decoy ng (peak centroiding, u ith different De Novo ) ee: 0.2 (1) nge: 4.0 (1)	charge decor parameter, ; Da • min. •	Peptide Score: Protein Score: Protein Score:	eisotope) Flow or se R Mn. 50 Mn. 50 Isation	lect a De No Seve as % Mac.	100 %	to start)
General Opti Validation v Perform on (If you want to Row Configuration thications all at PS(PS) level .mAQ ol at PS level .toX	Ions with reverse databa -the-fly proprocess. I abel Pree Sample Label Free Basic Options Mass Drar Taleran Reterition Tree Ra Upper Bound of Pre	se as decoy ng (peak centroiding, r ith different De Novo ) en 0.2 ( nge: 6.0 ( ecusor Charge:	barge decor parameter, p Da • me. • •	wolution, and d idease use Work defau Peptide Score: Protein Score: Votein Score: do normal	elsotope) Flow or se R	lect a De No Save as % Max. % Max.	100 %	to start)
General Opti Validation + Perform on (If you want to Flow Configuration flications el at PS(PS) level TEAQ el at PS(INS) el at PS(IN	ions with reverse databa h-the-fly preprocess do protein soarch w Label Free Sangle Label Free Basic Options Reservon Tolevan Reservon Tolevan Reservon Tolevan Reservon Tolevan Parameter Table	se as decoy ng (pesk-centroiding, i eth different: De Novo p eth in the second second second second nge (+0	barge decor parameter, p m. • •	wolution, and d idease use Work defau Peptide Score: Protein Score: I do normal	eisotope) Flow or st R Mn. 50 Isation	lect a De No	100 %	to start)
General Opti Validation + Perform on (If you want to Rear Configuration Microtions and at 105 level and at 105 level and at 105 level and at 105 level and at 105 level	ions with reverse databa h-the-fly preprocessi a do protein soarch w Label Free Basic Options Basic Options Reterition Tieleran Reterition Tieleran Reterition Tieleran Per anater Table	se as decoy ng (peak centroiding, 4 eth different De Novo p en: 0.2 () nge: 4.0 () Sangle Name	barge decor parameter, p Da • • • • •	Velution, and d please use Work defau Peptide Score: Protein Score: Ø do normal Pile Nar	eisotope) Flow or st k Min. 50 Min. 50 Isation	lect a De No Save as % Max. % Max. Protein ID	100 %	to start)
General Opti Valdation + Perform on (If you want to Plow Configuration Plow Configuration	In the reverse databases of the reverse o	se as decoy ng (peak centroiding, ( ikh different De Novo y en 0.2 ngit 4.0 songle 1.2	Da + mm. + Praction Number	Peptide Score: Ødea Peptide Score: Potein Score: Ø do normal	elisotope) Flow or se & Mn. 50 Mn. 50 Isation	Inct a De No Save as 5 Save as 76 Max. 76 Max. Protein ID From work/	100 %	to start)
General Opti Valdston + Perform on (If you wont to Reve Configuration Reve Configuration Reve Configuration Reve Configuration Reve at at MpR level 1527 et al.	Ions Ions Ion	se as decoy ng (peak-centroiding, 4 eth different De Novo eth different De Novo ngti 6.0 euror Charger Sangle Nane Sangle Nane Sangle 1 Sangle 2	barge decor parameter, p m. • • • • • • •	Velution, and d Joase use Work datas Paptide Score: Protein Score: do normal Pfle Nar UrgGan	elsotope) Flow or se Min. 50 Min. 50 Isation	ect a De No Save as 5 Save as 76 Max. 76 Max. 76 Max.	100 %	to start)

If it is a project for replicate experiments, you can click "Assign replicate" to add replicate information. Each label free quantification needs at least two samples and a replicate project contains at least four samples. You can also change the number of replicates. Currently the maximum number of allowed replicates is three.

After assigning replicates, PEAKS will generate parameter tables for each replicate automatically. The default setting is the same for each replicate and you can change the settings for a replicate by click the name of the replicates and modify the parameter table.

Click "OK" and then click "Start" in the workflow configuration panel. The following window will open:

Click "Start Jobs".

	nary			
Name	Refine Data	De novo	Peaks Search	Quantification
🖃 📲 selected data				
🔷 🗢 Sample 1	yes	yes	yes	yes
🛶 🏶 Sample 2	yes	yes	yes	yes
Start Jobs	Back	to Configura	ation Ca	ancel

#### 11.4.4 LABEL FREE RESULTS

Once the workflow analysis is complete, a label free quantification result node will appear on the project view.

You can double click on the label free node to open the result panel or right click on it to find more operations supported on a label free quantification result.

We support exporting the label free quantification results to Excel or HTML file by right clicking the result node and choosing the corresponding function. Please refer to section <u>15 Exporting Data/Reports and Printing</u> for details. We also support changing the normalization factor in calculating the protein ratio. After clicking "Normalization settings" in the right click pop up menu, the following dialog will pop up:

If you uncheck the "do normalization" option in configuring label free quantification, the protein ratio will be calculated from peptide ratios without normalization. Otherwise, we will normalize it and the normalization factor will be displayed. You can also set the normalization factor manually by clicking the "Manually Normalize Peptide Ratios" and input the ratios

Normalization Settings	23
Our Unormalize Peptide Ratios	
Automatically Normalize Peptide Ratios	
Manually Normalize Peptide Ratios	
1.0 : 1.0	
0	K Cancel

in the text field. The format of ratios should be digital numbers separated by colon and the number ratios should be the same as the number of samples in the quantification.

After double clicking the result panel, the quantified proteins, supporting peptides of each protein and peptide features in the spectrum from each sample will be displayed in the result panel. The quantified proteins will appear in the top panel, with homologous proteins clustered together. The ratio of Sample 1: Sample 2 appears in the "Ratio" column and the standard deviation of Sample 1: Sample 2 appears in the "SD" column.

In the example below the standard deviation is 0 as there is only one supporting

Q LA											
View	Accession	ID	Mass	Score 🗸	Coverage	Matched	Description	Ratio	SD	Marked	<b>₽</b>
.⊑	🖃 🌑 Quantification Result										-
L, L, L	💭 P27824 CALX_HUMAN	17	67,568.453		3.37		Calnexin precurso	1.00:1.12	0.00:0.00		
	🖨 🌑 P08238 H590B_HUMAN	5	83,264.234	0.6	4.28	4	Heat shock protei	1.00:1.11	0.00 : 0.04		-
	Q76LV1 H590B_BO	4	83,253.203	0.6	4.28	4	Heat shock protei	1.00:1.11	0.00:0.04		=
	Q9NYF8 BCLF1_HUMAN	19	106,122.172	0.4	1.52	3	Bcl-2-associated tr	1.00:1.07	0.00 : 0.01		

peptide for the protein shown.

The supporting peptide is shown under the "Peptides" tab. The retention time is shown for the specific peptide as well as the peptide ratio from Sample 1: Sample 2.

Click on the "+" beside the "Outlier" folder to see the peptides that were not included in the ratio.

To see which peptides were used to identify the protein during the PEAKS protein ID

Sequence	Peaks QScore	M/Z	Z	Retention Time	Ratio	E
🖨 퉲 Unique Peptides						
QKSDAEEDGGTVSQEEEDR		730.289		[33.31, 34.35]	1.00 : 0.75	
SDAEEDGGTVSQEEEDR	0.2	966.85284	2	[33.33, 34.06]	1.00:0.70	
QKSDAEEDGGTVSQEEEDR	0.2	1134.9127	2	[32.62, 33.35]	1.00:0.62	
QKSDAEEDGGTVSQEEEDR	0.2	756.9444	3	[32.62, 33.46]	1.00:0.68	
QKSDAEEDGGTVSQEEEDR	0.2	1094.9296	2	[33.33, 34.38]	1.00:0.70	
Outlier						
Multi Cluster Supports						

search, select the "Coverage" tab. The entire sequence of the protein is shown and the matching peptides are highlighted in blue. In this example the total matched part accounts for 9.392% of the protein. This information can be found in the "Coverage" column in the "Protein View" panel.

The features chart will appear by default in the bottom panel:

To see exactly where the selected supporting peptide corresponds to the protein sequence, select the "Peptide detail" tab. The entire sequence of the protein is shown and the selected supporting peptide is highlighted in blue.

Clicking on the "Quantification" tab will display a 2D view of the features. Move your cursor around the map to display the m/z ratio and retention time.



When there are more than nine samples in the project, we will display features of the first nine samples as default. If you want to display features of specific samples, click the triangle on the left of the

quantification panel and a selection menu will pop up as follows. You can check the samples you are interested in viewing.





Click on the "3D View" tab to display a 3D View of the peptide features for sample 1 and sample 2. Intensity is displayed on the y-axis, m/z on the x-axis and retention time on the z-axis. Click and move the cursor to rotate around the image. Notice that as you move one sample image the other sample moves to the same location.





# 12 CREATING A HIGH-THROUGHPUT WORKFLOW

For your convenience, PEAKS provides workflows for protein identification, quantification and inChorus search (multi-engine protein ID).

#### 12.1 IDENTIFICATION WORKFLOW

Click the workflow icon on the toolbar  $\bigotimes$  and select "identification", the identification workflow configuration window will appear:

Click "Select Data" to select the data you wish to perform identification analysis. Select a data file, and click "Add to Right" to transfer the samples/files to the "Selected Data" panel on the right hand side. Use the "Remove" and "Clear" buttons to remove the selected file, respectively from the "Selected Data" panel. Click "OK" to proceed to the next step.

Work Flow		23
1.	Select Data	unfinished
2.	K Refine Data	
3.	De novo	
4.	Peaks Search	
5.	RTM Finder	
6.	Spider Homolog	
	S	tart Cancel

Al Data Ci/FEACS_Studio_5.2/./derbyServer/ServerD6/test D- [] Sample 1 Ci/FEACS_Studio_5.2/./derbyServer/ServerD6/test D- [] Sample 1 Ci/FEACS_Statistics model	Sencted Data     Sencted Data     Sence Data     Sence 1     Sence 1     Sence 1     Sence 1     Sence 1     Sence 1
All Samples All Fractions Add to Right	Remove

Check the boxes beside the functions that you would like to perform within the workflow. Note that PEAKS requires de novo sequencing results to perform Protein ID.

Then, you can define the parameters for data refinement, de novo, PEAKS search, PTM Finder and SPIDER. Please require more details on setting up the parameters

refer to the chapters on each individual function if you require more details on setting up the parameters.

# Note that PTM Finder and SPIDER are optional functions. You can uncheck them if you do not want to perform those functions.

#### 12.2 QUANTIFICATION WORKFLOW

The quantification workflow is similar to the identification workflow. Except after Protein ID, you can define quantification parameters to perform labeled or label free quantification.

i. [	🕤 Select Data	unfinished
2.	K Refine Data	
3.	Le novo	
4.	Peaks Search	]
5.	Q Quantification	]
		Start Cancel

#### 12.3 INCHORUS WORKFLOW

inChorus workflow is similar to identification workflow except that at the third step, you can specify inChorus parameters to start a inChorus search.

Work Flow		E
1.	😽 Select Data	unfinished
2.	🔍 Refine Data	
з.	InChorus	

#### 12.4 BATCH WORKFLOW

In some situations, you may have many data sets that you wish to process all at once, with the same parameters. PEAKS allows you to do this type of work using a workflow. By setting up different workflows, you can start a batch process of several projects and perform de novo, protein ID, SPIDER searches, as well as quantification without having to run each step individually.

It is important to note that all the files you load in a single workflow will be processed in exactly the same way, using exactly the same parameters. If you want to do some differently than others, you must set up separate workflows.

Click "Select Data" button.

Only projects that are open in the "Project View panel" can be selected for analysis with the workflow function. To select which files/samples you would like to analyze, select the individual file/sample or click the "All Samples" or the "All Fractions" buttons and then click "Add to Right" to transfer the samples/files to the "Selected Data" panel on the right hand side. Use the "Remove" and "Clear" buttons to remove selected files/samples or all files/samples, respectively from the "Selected Data" panel. Click "OK" to proceed to the next step.



# **13 FILTERING YOUR RESULTS**

PEAKS 5.2 provides you with an exhaustive list of all proteins and peptides that can be identified in a sample. However, as many researchers have their own criteria of what information is required in a report as well as what is an acceptable result, PEAKS 5.2 provides the necessary tools that enable filtering out the less critical information, leaving just the essentials.

#### **13.1 SETTING FILTER PARAMETERS**

Double-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 will appear:

🛃 Filter		×
Tools	Filter Parameters Save Parameters	
✓. Result Filtering	Possible Filters	Selected Filters
	Do Novo Fiters Poptide Fiters Protein Fiters	De Novo Fiters     Peptide Fiters     Protein Fitters
	Parameter Options Set saved as default Default Parameters:	Edt Filter
	OK Cancel	Help



PEAKS will automatically create a new filtered results node in the project view that will save your filter choice every time you click "OK".

Note: The filter node relies upon the original result so DO NOT DELETE the original result in order to keep all associated filtered result nodes.

#### 13.1.1 POSSIBLE FILTERS/ SELECTED FILTERS/ EDIT FILTER

The filters are grouped into three basic types. Each type identifies the level on which the filter will be performed:

- de novo filters act to remove proposed de novo sequences
- Peptide Filters act to remove peptides found in the database from the report
- 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. Here you can see a large variety of built-in filters:



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 for the filter in the "Edit Filter" frame, click and drag the filter into the "Selected Filter" list on the right hand side. Ensure that you move the filter to the appropriate de novo, peptide or protein node in

the selected filters pane. Click "OK" to apply the filter(s) that you have selected to the current file.

You can add multiple filters by simply repeating the process outlined above. In this way it is also possible to have two filters with the same property; i.e. we can set a range of protein mass by applying one filter on the upper bound of the mass and adding a second filter to be the lower bound of the mass.

**Example**: Let's say that you want to show only proteins with more than one high scoring peptide (greater than 60%) 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. Now click and drag "Score filter" from the "Possible Filters" frame to the "Selected Filters" frame.

Save Parameters

De Novo Filters

Peptide Filters

Selected Filters

• Score (%) Protein Filters

Edit Filter	Score (%):
Filter Pro Score(%)	einID peptides based on their
	60 🚔
Options	:
💿 Grea	er than
💿 Lesse	r than
💿 Not e	qual to



Filter Parameters

Possible Filters

le Peptide Filters

Sequence filter (Basic)

Sequence filter (Adv.

Score (#) filter
 m/z filter
 Charge filter
 Mr(calc) filter
 RT(calc) filter
 n-value filter

Filter sets can be saved and re-used between sessions by clicking the "Save Parameters" 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 drop-down 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.

Peptide filters and protein filters can be applied to a protein id result at the same time, potentially making filtered results confusing. The basic principle for two different types of filters working together is:

- If a protein is filtered out by the given protein filter, all of its unique supporting peptides will also be filtered out
- If all of the supporting peptides of a protein have been filtered by the peptide filter, this protein will also filtered out.

We will use some samples to illustrate the usage of complex filters:

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.

• 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".



# **14 COMPARISON FUNCTIONS**

#### 14.1 OVERVIEW

In liquid chromatography-mass spectrometry (LC-MS) based proteomics, multiple samples from different groups are analyzed in parallel. Tools that access the quality of proteomics data based on sound statistical principles are needed in this field. In PEAKS 5.2, comparison functions are provided in three levels:

- Assess the reproducibility of MS data from technical replicates
- Perform compare analysis of peptide and proteins
- Assess the reproducibility of protein quantification from biological/technical replicates

This chapter is organized to first introduce how to create a replicate project. The replicate analysis of MS data comparisons and label free quantification are done together and so each function will be introduced together in one section. Secondly, the comparison function of peptide and proteins will be given later in this chapter.

#### 14.2 CREATING REPLICATE PROJECT

Replicate analysis of sample data and label free quantification results in PEAKS 5.2 can be done by assigning replicate samples when initially setting up the project. Alternatively, the replicate information can be assigned to samples and fractions when you set parameters for label free quantification.

As show in the screenshots below, if you check "Replicate" in the sample details and select 1, the selected sample will be assigned to the first replicate. After adding replicate information, a label will appear before the samples name

indicating the replicate and the index of the sample. For example, R1S1 means the first sample in replicate 1. In this way, the samples with the same index (S1) of different replicates (R1, R2, etc.) should be technical or biological replicates of each other.

Currently, three replicates are allowed in a replicate project and the number of samples within different replicates should be the same. Different replicates can also be distinguished from the color of icons associated with each sample.

			1
New Project			
Project Name	Replicate Analysis		
Project Location	D:\workspace\derbyServer\serve	erDB	Browse
Data Files		Sample Details	
Replicate Analysis	1	Replicate	Sample Name
C LFQSample: Add data file	1.mzXML	1 -	Sample 1
England	2.mzXML	1: FT-trap	
R2S1: Sample 3	<u>:::::::::::::::::::::::::::::::::::::</u>		
Add data file	I - Copy.mzXML		
LFQSample:	2 - Copy.mzXML		
Delete Up	Down Add Sample	Sort by Replicate	OK Cancel

### 14.3 COMPARE DATA AND LABEL FREE QUANTIFICATION

Replication is a standard for validating proteomics data and findings. The samples utilized within an experimental design often include technique and biological replicates. In PEAKS 5.2, we provide powerful replicate analysis tools for data and label free quantification results.

#### 14.3.1 PARAMETER SETTING

After acquiring label free quantification results, right click on the project node and select "Replicate Analysis".



The following dialog will appear:

The analysis consists of replicate data comparison and replicate result comparison. We support analysis on at most two replicates in data comparison and three replicates in result comparison.

For replicate data comparison, you need to select one data file you want to compare from each replicate.

For replicate result comparison, you need to select one label free quantification result for each replicate and two samples you want to

Refinement         Image: Case 1: Spike5_040806_1.RAW         Image: Case Analysis         Image: Spike5_040806_1.RAW         Image: Spike1_040806_1.RAW         Image: Spike2_040806_1.RAW         Image: Spike2_040806_1.RAW         Image: Spike2_040806_1.RAW         Image: Spike3_040806_1.RAW         Image: Spike3_04		🛛 Replicate Da	ata Comparision				
Refinement <ul> <li>Spike5_040806_1.RAW</li> <li>Spike5_040806_1.RAW</li> <li>Spike0_040806_1.RAW</li> <li>Spike0_040806_1.RAW</li> <li>Spike0_040806_1.RAW</li> <li>Spike1_040806_1.RAW</li> <li>Spike1_040806_1.RAW</li></ul>		Replicate 1 : Spi	ke5_040806_1.RAW	📝 Replicate 2 : Spik	e5_040806_2.RAW	Replicate 3 : Spike	5_040806_3.RAW
cate Analysis       Spike0_040806_1.RAW       Spike0_040806_2.RAW       Spike0_040806_3.RAW         ovo       Spike1_040806_1.RAW       Spike1_040806_2.RAW       Spike1_040806_3.RAW         S Spike1_040806_1.RAW       Spike2_040806_2.RAW       Spike2_040806_3.RAW         S Spike1_040806_1.RAW       Spike3_040806_2.RAW       Spike3_040806_3.RAW         S Spike1_040806_1.RAW       Spike3_040806_2.RAW       Spike3_040806_3.RAW         S Spike1_040806_1.RAW       Spike4_040806_2.RAW       Spike4_040806_3.RAW         Spike5_040806_1.RAW       Spike5_040806_2.RAW       Spike6_040806_3.RAW         Spike6_040806_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike1_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike1_040806_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike1_040806_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike1_040806_1.RAW       Spike1       Spike1         Spike1_0	Refinement	Spike5_040806	_1.RAW	Spike5_040806	_2.RAW	③ Spike5_040806	_3.RAW
wvo       Spike 1_040806_1.RAW       Spike 1_040806_2.RAW       Spike 1_040806_3.RAW         3 Search       Spike 3_040806_1.RAW       Spike 3_040806_3.RAW       Spike 3_040806_3.RAW         3R Search       Spike 1_040806_1.RAW       Spike 3_040806_3.RAW       Spike 3_040806_3.RAW         3 Search       Spike 1_040806_1.RAW       Spike 3_040806_3.RAW       Spike 4_040806_3.RAW         Spike 1_040806_1.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW         Spike 1_040806_1.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW         Spike 1_040806_1.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW         Spike 1_040806_1.RAW       Spike 4_040806_2.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW         Spike 1_040806_1.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW       Spike 4_040806_3.RAW         V       Replicate 1:       LABEL FREE 82 * <td< td=""><td>ate Analysis</td><td>Spike0_040806</td><td>_1.RAW</td><td>Spike0_040806</td><td>_2.RAW</td><td>Spike0_040806</td><td>_3.RAW</td></td<>	ate Analysis	Spike0_040806	_1.RAW	Spike0_040806	_2.RAW	Spike0_040806	_3.RAW
vo       Spike2_040806_1.RAW       Spike2_040806_2.RAW       Spike3_040806_3.RAW         Search       Spike3_040806_1.RAW       Spike3_040806_2.RAW       Spike3_040806_3.RAW         R Search       Spike4_040806_1.RAW       Spike4_040806_2.RAW       Spike4_040806_3.RAW         Spike5_040806_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike5_040806_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike5_040806_1.RAW       Spike7_040806_2.RAW       Spike6_040806_3.RAW         Spike6_040806_1.RAW       Spike8_040806_2.RAW       Spike6_040806_3.RAW         Spike6_040806_1.RAW       Spike8_040806_2.RAW       Spike6_040806_3.RAW         Spike6_040806_1.RAW       Spike8_040806_2.RAW       Spike6_040806_3.RAW         Spike6_040806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         Spike1_010806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         Spike2_010806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         Spike1_1       LABEL FREE 83         Y Replicate 1:       LABEL FREE 84         Sected :       Sample 1       Sample 13       Sample 19 / Sample 19 / Sample 19 / Sample 19 / Sample 20         Sample 3       Sample 13       Sample 21       Sample 21       Sample 21       Sample 23       Sample 23		Spike 1_040806	_1.RAW	Spike 1_040806	_2b.RAW	Spike 1_040806	_3.RAW
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R Search       Spike4_040806_1.RAW       Spike4_040806_2.RAW       Spike4_040806_3.RAW         Spike6_040806_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike7_040806_1.RAW       Spike7_040806_2.RAW       Spike6_040806_3.RAW         Spike8_040806_1.RAW       Spike7_040806_2.RAW       Spike6_040806_3.RAW         Spike8_040806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         Spike8_040806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         Spike8_040806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         VReplicate 1:       LABEL FREE 82 V Pepicate 2:       LABEL FREE 83 V Pepicate 3:       LABEL FREE 84         Selected :       Sample 1 / Sample 2       Selected :       Sample 10 / Sample 10 / Sample 11       Selected :         VSample 1       VSample 10       VSample 10       VSample 20       Sample 19 / Sample 20       Sample 13         Sample 3       Sample 13       Sample 21       Sample 21       Sample 21         Sample 4       Sample 14       Sample 23       Sample 24       Sample 24	Search	Spike3_040806	1.RAW	Spike3_040806	2.RAW	Spike3_040806	_3.RAW
Spike6_040806_1.RAW       Spike6_040806_2.RAW       Spike6_040806_3.RAW         Spike7_040806_1.RAW       Spike7_040806_2.RAW       Spike7_040806_3.RAW         Spike8_040806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         Spike8_040806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         V       Replicate Result Comparision       V         V       Replicate 1:       LABEL FREE 82 V       V Replicate 2:         Selected :       Sample 1 / Sample 2       Selected :       Sample 10 / Sample 11         V       Sample 1       V Sample 10       V Sample 19 / Sample 19         V       Sample 1       Sample 11       V Sample 12         Sample 3       Sample 13       Sample 21         Sample 4       Sample 13       Sample 23         Sample 5       Sample 15       Sample 24	8 Search	Spike4_040806	_1.RAW	Spike4_040806	_2.RAW	Spike4_040806	_3.RAW
oder       Spike7_040806_1.RAW       Spike7_040806_2.RAW       Spike7_040806_3.RAW         Spike8_040806_1.RAW       Spike8_040806_2.RAW       Spike8_040806_3.RAW         V       Replicate Result Comparision         V       Replicate 1:       LABEL FREE 82 V       V Replicate 2:       LABEL FREE 83 V       Replicate 3:       LABEL FREE 84         Selected :       Sample 1/Sample 2       Selected :       Sample 10 / Sample 11       Sample 19 / Sample 19         V       Sample 1       V Sample 10       V Sample 19       Sample 19 / Sample 10       Sample 19 / Sample 19         Sample 3       Sample 11       V Sample 11       V Sample 20       Sample 12       Sample 21         Sample 4       Sample 13       Sample 23       Sample 14       Sample 23       Sample 24         Sample 5       Sample 15       Sample 24       Sample 24       Sample 24	Cocorer	Spike6_040806	_1.RAW	Spike6_040806	_2.RAW	Spike6_040806	_3.RAW
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Image: Sample 1       Image: Sample 1       Image: Sample 2       Image: Sample 2       Image: Sample 1       Image: Sample 2		Spike8_040806	_1.RAW	Spike8_040806	_2.RAW	Spike8_040806	_3.RAW
Y Sample 1       Y Sample 10       Y Sample 19         Y Sample 2       Y Sample 11       Y Sample 20         Sample 3       Sample 12       Sample 21         Sample 4       Sample 13       Sample 22         Sample 5       Sample 14       Sample 23         Sample 6       Sample 15       Sample 24		Replicate 1 :	LABEL FREE 82   Sample 1 / Sample 2	Replicate 2 : Selected :	LABEL FREE 83 -	Replicate 3 : Selected :	LABEL FREE 84 Sample 19 / Sample
V Sample 2       V Sample 11       V Sample 20         Sample 3       Sample 12       Sample 21         Sample 4       Sample 13       Sample 22         Sample 5       Sample 14       Sample 23         Sample 6       Sample 15       Sample 24		Sample 1		Sample 10		Sample 19	
Sample 3         Sample 12         Sample 21           Sample 4         Sample 13         Sample 22           Sample 5         Sample 14         Sample 23           Sample 6         Sample 15         Sample 24		Sample 2		🔽 Sample 11		Sample 20	
Sample 4         Sample 13         Sample 22           Sample 5         Sample 14         Sample 23           Sample 6         Sample 15         Sample 24		Sample 3		Sample 12		Sample 21	
Sample 5         Sample 14         Sample 23           Sample 6         Sample 15         Sample 24		Sample 4		Sample 13		Sample 22	
Sample 6 Sample 15 Sample 24		Sample 5		Sample 14		Sample 23	
		Sample 6		Sample 15		Sample 24	

compare. Once you select a sample, all the samples with the same index in other replicates will be selected automatically.

#### 14.3.2 REPLICATE ANALYSIS RESULT

When a replicate analysis is done, a "replicate analysis" node will be added to the project and the results panels will open directly. The analysis will include the following information:

Replicate Data Comparison:

- Feature Comparison
- Feature Venn Diagram

Replicate Result Comparison:

- Peptide Scatter Plot
- Peptide Venn Diagram
- Protein Q-Q plot

Feature Comparison Scatterplot:



The feature comparison scatter plot represents each feature vector which consists of two features detected in the two data files you want to analyze and aligned in the label free quantification. The x-axis is the log intensity of the feature detected in the first data file and the y-axis is the log intensity of the feature detected in the second data file. The Pearson Correlation Coefficient is calculated and listed under the chart. The standard box plot is shown on the right side of scatter plot.

#### Feature Venn diagram:

The feature venn diagram is a standard venn diagram showing the number of common peptide features and unique peptide features of the two data files.





The peptide scatter plot represents the peptides quantified in two label free quantification results in comparison. The x-axis is the ratio of the peptide of label free quantification result 1 and the y-axis is the ratio of the same peptide relative intensity ratios in corresponding samples of label free quantification result B.



Peptide Venn diagram:

The peptide venn diagram is a standard venn diagram showing the number of quantified common peptides and unique peptides of label free quantification results in comparison

Q-Q Plot

The protein Q-Q plot is a standard quantile plot of protein ratios from selected samples of label free quantification results in comparison. The ratios of the proteins in the first sample is plotted against the ratios of the proteins in the second ratio, both in ascending order of size, and scaled from 0 to 100. In the ideal case, both replicates should result in the same ratios for the proteins, and thus the expected result is represented by the diagonal line in red.

#### **14.4 COMPARE PROTEIN ID RESULTS**

In PEAKS 5.2, we support comparisons on at most three PEAKS Protein ID results (including filtered results) in one project. To do such a comparison, select those PEAKS Protein ID nodes and right click. Click on "Compare Results" and the comparison will be done automatically.

#### 14.4.1 COMPARISON RESULT

After comparison is finished, a comparison node will be added to the project as shown in the following picture.

D:/workspace/derbyServer/serverDB/New Project 2
 Compare run: 1,3
 DENOVO 1
 PEAKS 1
 LABEL FREE 1
 PEAKS 3
 LFQSample 1.mzXML
 DATA REFINE 1
 Sample 2
 LFQSample2.mzXML
 ATA REFINE 2

The result panel will be opened automatically after completing the comparison. Since the comparison run is done on the fly it won't be saved, it is suggested to export the results before closing the result panel. The details of exporting will be given in the next subsection.

The result consists of three parts: peptide comparison, protein comparison and statistical charts. Below is an outline of each.





#### 14.4.1.1 PEPTIDE COMPARISON

All the peptides identified in the two PEAKS Protein ID searches are displayed in the table. We show m/z, retention time, peptide score, charge and whether there are multiple hits for each peptide. The 'cover map' is a quick graphical illustration of the presence of the given peptide in one or both protein ID results. A solid icon indicates a successful detection of the peptide.

You can also select to show only the common peptides of those protein ID results, or the unique peptides of each protein ID result by changing the display settings at the bottom of the panel.

We also provide filters on the peptide comparison results. After inputting the PEAKS score threshold on each protein ID result and clicking "Apply Threshold" button, those peptides below the threshold will be filtered out.

The following screenshot is a typical peptide comparison result:

DLSDEKDSC(+57.02)SR		C			PEAKS 1					PEAKS 3		
DLSDEKDSC(+57.02)SR	Jence	Cover Map	M/Z	RT	Score	Z	MULTI-HIT	M/Z	RT	Score	Z	MULTI-H
								656.27	32.35	7.07	2	false
AEKDEPGAWEETFK								818.87	34.94	7.07	2	false
SAPLGPVAPTR								533.31	32.71	49.08	2	false
EYSHGYMDK(+6.02)								568.22	34.55	7.07	2	false
SAGC(+57.02)AMNDYADR								665.77	33.96	7.07	2	false
EPGEAAAEGAAEEARR								807.36	32.24	20.27	2	false
NGGDPDIYAK								525.23	35.98	19.4	2	false
QKMTM(+15.99)SDLSDR								664.28	35.63	17.21	2	false
FYGVELQGK(+6.02)DC(+57.02)AIIGASMVVGK(+6.0	PAALLLLNENATISVC(+57.02)HIFTK(+6.02)							1556.18	35.49	23.32	3	false
LSGSSEDEEDSGKGEPTAK(+6.02)								643.62	35.36	18.88	3	false
SYQNSPSSDDGGGSGSR								829.36	36.35	7.07	2	false
GHM(+15.99)EFSSPR								532.23	34.46	36.48	2	false
VDGSLAVSR			452.2	31.75	17.57	2	false					
WM(+15.99)PGVSM(+15.99)VTEEDR								784.85	32.85	7.07	2	false
MGDEMDAM(+15.99)IPER				-				705.81	34.76	35,48	2	false
STSYGAELAR				-	-		-	536.75	34,43	45.51	2	false
FTVRDM(+15.99)K			453.76	35.1	24.65	2	false	453,76	35.1	82.12	2	false
EM(+15,99)SNTWC(+57,02)TPLR						-		476.87	36.33	26.39	3	false
AM(+15.99)MM(+15.99)K				-				643.28	36.33	21.88	1	false
C(+57.02)DNITDC(+57.02)ADGSDEK						_		800.32	32.77	7.07	2	false
C(+57.02)DR(15C(+57.02)CAWIYWEEDSCCDAM(+15	ID)EVK(16.02)					-		656.53	36.09	35.55	4	true
TVAC(±57.02)GCTK(±6.02)	5)244(10:02)				-	-		400.19	34.65	47.78	2	falco
M(+1E 00)0DETLDCED					-			529.74	26.45	40.91	2	folco
V(+E 02) NEEDAACECCD								721 22	22.44	60.00	2	falco
HTC/MVCMCOK								572.33	22.99	70.10	2	falco
NEESDASDEAEEK(+6.02)EAK(+6.02)								997 90	22.90	10.99	2	falco
NEESPASDEAEER(+0.02)EAR(+0.02)			542.22	26.49	25.62	2	false	007.09	52.44	19.09	2	Idise
			491.90	30.40	23.62	2	false	491.90	24.6	E2.4E	2	false
			401.09	54.0	0.00	3	laise	410.16	25.02	33.45	2	false
LM(+15.99)AEDCEG1K				_				410.10	33.03	22.23	2	false
M(+15.99)VMDVVR								497.25	32.21	24.63	2	false
DAM(+15.99)PSPDMGGMGGMGGMGF				_		_		606.89	35.67	7.07	2	false
155AFNVESC(+57.02)5L1PG1M(+15.99)K								009.03	35.73	/.0/	2	Taise
C(+57.02)RQQQQSGSSTK								465.54	31.65	45.83	3	raise
DEEESADSKDIR								697.3	36.16	7.07	2	raise
NVYEQQK(+6.02)YYGM(+15.99)SSR								887.89	32.44	7.07	2	false
DENSVELIMAEGPYK								841.87	32.61	7.07	2	false
ALM(+15.99)ARC(+57.02)C(+57.02)R				_				527.23	31.73	26.83	2	false
EGNM(+15.99)C(+57.02)GDVDFDGVK				_				779.82	31.54	25.81	2	true
QC(+57.02)DDGTC(+57.02)IAEHK								717.29	35.67	7.07	2	false
LESENDEYER								642.26	32.75	35.9	2	false
								624.79	34.02	25.4	2	false
NISEGRSNESR								412.21	33.16	26.26	2	true

#### 14.4.1.2 PROTEIN COMPARISON

The top proteins identified in the PEAKS Protein IDs are displayed in the table. The display setting, score filter and cover map function the same as in the peptide comparison frame. The following information is also displayed for each protein:

Score: PEAKS protein score

#Spec: the number of spectrum on which this protein has been detected.

#Pep: the number of supporting peptides of the protein

#Uniq: the number of unique peptides of the protein

%Spec: the ratio of detected peptides to the theoretical numbers

%Cov: the peptide coverage of the protein

The following screenshot is a typical results tab for protein comparisons:

Burkelo III				PEAKS 1						PEAKS 3			
Protein_ID	Cover Map	Score	#Spec	#Pep	#Uniq	%Spec	%Cov	Score	#Spec	#Pep	#Uniq	%Gpec	%Co
P60035jACTC_RAT		80.53	4	3	2	300	0.49	1.0.00		1.1		100 m	1000
P1427290LKB1_RAT								17.04	1	1	1	2.27	1.1
Q8N954(CCD75_HUMAN		10					1	6.09	3	3	3	13.04	13.69
017320 ACT_CRAGE		80.53	4	3	2	300	0.51						
A4Y138 ATPG_PSEMY								35.8	2	2	2	10	7.32
P60710(ACTB_MOUSE		80.53	4	3	2	300	8.53					1.0	
P10983[ACT1_CAEEL		80.53	4	3	2	300	8.51	1.000	1.00	1.00		100	1.1.1.1
P\$3459 ACT6_DIPDE				1.1	-	1	-	380.52	3	2	0	8.33	5.63
Q758N2(ATG13_ASHGO		6.33	1	1	1	100	2.12					-	
P05419 CALN_CHICK		60.4	1	1	1	100	6.87	61.58	1	1	1	11.11	6.87
Q88AF9]MTGA_PSESM								21.17	1	1	1	5.56	5.93
Q\$\$3U6JACT22_DICDI		Sec. 1				1		95.05	4	3	0	12.5	8.51
P68133JACTS_HUMAN		80.53	4	3	2	300	3.49	1000	1.	1. 11		1000	1.0
22UFN7(ATG17_ASPOR		9.47	3	2	2	200	3.45	47.28	2	1	1	2.63	1.82
OP12KSUACTB SIGHT		30.53	4	3	2	300	8.53						
29475IN051 HLMAN			T.		-	-		15.00	2	1	1	0.97	0.63
D14319ICLES SCHPO								11.7	3	3	3	6.25	3.69
D9NYZ3IGTSE1 HLMAN	110		-		-	-	-	18.05	1	1	1	2.08	1.11
P15475IACTE XENBO		80.53	4	3	2	300	8.51		-	-			
DODDZIACTS ORVLA	80	00.53	4	3	2	200	0.49						1.0
D09711IVA/E SCHED			-	-	- F			44.32	2	2	2	2.99	2.08
P53471JaCT2 SCHMA	80	80.53	4	3	2	300	8.51			-	- <u> </u>		
DED158704VA SCHPD	1000	100.00			- f		1.00	15.61	2	2	3	5.21	6.26
PS3501IACT3 DROME		40.53	4	3	2	300	8.51				1		100
P84185IACT5C ANOGA		80.53	14	3	6	300	8.51	-	-			-	
05-Kix7laCT10 DICDI	0.00			5.				95.05	4	3	0	12.5	8.51
PASERTIACTS EACDO	80	80.53	4	3	2	300	8.51			-		1	
P68753 MATK PAECA	110		1	-	- F			5.20	1	1	1	3.12	2.82
D8Z365IENDP1 SALTI		8.03	2	2	2	200	2.49	48.02	4	3	3	4.55	3.86
ASILCS MOH AZOCS	[100		1	10	1			3.56	1	1	1	5.26	2.49
PROGRACTS DROST		81.61	5	4	3	400	13.56			-	-		-
MANTS RANT	80	80.53	4	3	2	300	8.49	-					
088778/BSN RAT	08				- f			56.29	6	5	1	2.18	1.35
0624078PEG MOUSE		5.32	3	3	3	300	1.1	38.84	4	3	3	1.48	0.89
DIRAY 18POC2 SOVEN		9.66	3	2	2	200	1.23	46.40	2	1	1	0.97	0.58
P63260 ACTG MOUSE		80.53	4	3	2	300	8.53	The Contemporate State		-			
09Y707IACT2 SUB0	80	80.53	4	3	2	300	8.53					1072	1.1
P4678838118 STRCO	00		1		1		1	38.09	1	1	1	9.09	8.66
DRULTARIZGA HUMAN		11.77	3	3	3	300	1.54	6.64	2	2	2	1.69	0.85
007903IACTC STRPU	80	90.53	4	3	2	200	0.51				- F		
P53498IACT CHLRE				-	6		-	95.05	4	3	0	12.5	8.49
DIAWOSIAROC RUBID	0.00		-		1			15.06	1	1	1	3.57	2.31
0627401ACTA RABIT	80	10.53	4	1	2	300	11,49						-
kore threshold for different n	ins:		in in interio		( Justice								
Score threshold for different n PEAKS 1 [14 Apr-10 22:44]:		PEAKS 3 (28-Apr	-10 13:48]:		Apply T	hreshold							

#### 14.4.1.3 STATISTICAL CHARTS:

PEAKS 5.2 provides a number of statistical charts which are able to be exported for use in publications. The peptide score distribution, protein score distribution, peptide number venn diagram and protein number venn diagram for users to validate their results.



#### 14.4.2 EXPORTING COMPARISON RESULTS

To export the comparison results of PEAKS Protein IDs, please right click on the comparison run node and choose to export to Excel/HTML file. Here you can choose image quality and filter the content you want to export.

D:/workspace/derbyServer/s	erverDB/New Project 2_vers	Export Options
Compare run; 1,3 DENOVO 1 PEAKS 1 LABEL FREE 1 EAKS 2	Combine Engines Compare Results Perform Filtering	Export peptide result     Export protein result     Export statistics grap
PEARS 3     P	Export PepXML Export Excel Export Html Export Statistics Graph Set Default Data Refine	Image Options Scale: 100 T
CrbiSample .mzXML     OrbiSample .mzXML     OATA REFINE 1     OATA REFINE 1     OATA REFINE 4	Delete Result Hide Result	

Expor	: peptide result t protein result	
Expor	t statistics graph	
Image Opt	ons	
Scale:		
File For	mat: png vidth: 800 Height: 600 v	
Save As:	compare result	Browse
	OK Cancel	H

# Chapter 15

# **15 EXPORTING DATA/REPORTS AND PRINTING**

PEAKS 5.2 allows you to create reports to share with collaborators, colleagues and clients. The reports are available in HTML or Microsoft Excel (.xls) formats and largely follow a 'What You See Is What You Get' philosophy while still adding information required to meet MCP (Bradshaw et al. 5 (5): 787. 2006) requirements. It is important that we complete results filtering and toggling columns before exporting a report.

Protein view exporting for PEAKS Protein ID and PEAKS LFQ both now have MCP exporting modes that attempt to collect as much information as possible to fit the MCP guidelines and make publication easier.

# 15.1 EXPORT SAMPLE DATA

Data can be exported to a number of file formats including .mzxml, .mgf, DTA, and PKL. To do so, right click on the data file that you wish to export.

For example, 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:

K Export mzXML File	<b>×</b>
Start RT:	End RT:
Save as:	Browse
ОК	Cancel



Enter the starting and ending retention times in the appropriate boxes. Then click the "Browse" button to select a destination to save your file.

#### 15.2 EXPORT RESULTS OF A FRACTION IN EXCEL OR HTML FORMAT

To export the results for a selected data file, right click on the data file and select "Export Result file". All of the PEAKS results file generated for the selected data file will be displayed in Excel or HTML format. This will create an output with the highest scoring result for all search engines sorted by spectrum.

#### **15.3 EXPORT RESULTS IN EXCEL OR HTML FORMAT**

In PEAKS 5.2, all the de novo results, database search results, including PEAKS Protein ID, PTM finder, Spider, other search engines from inChorus, and quantification results can be exported to Excel or HTML Format.

#### 15.3.1 EXPORT DE NOVO RESULTS

To export the de novo results, right click on a de novo result node and choose "Export HTML" or "Export Excel"



40 50

The following dialog will appear:

If the 'export sequence tags' option is selected, you can set a threshold for exporting your de novo sequences. Essentially, de novo residues with local confidences scores lower than the threshold will be displayed as mass gaps while de novo residues with local confidence scores higher than the threshold will be displayed as expected.

A typical exporting file of de novo results contains all the de novo peptide information: id, sequence, TLC, ALC, rank, m/z, charge (Z), mass, file, retention time, scans, quality and fragmentation mode. The fraction information and parameters during the de novo run will append at the end of the file.

Export Excel Re

ect the Type of Results to Expor Export Sequence Tags Score Threshold:

Select the Export Destination xls File: D:\workspace\

10

D:\workspace\New Project 5\_DENOVO\_3.xl

#### 15.3.2 EXPORT DATABASE SEARCH RESULTS

To export the database search results are similar, only with slight differences in the results contents. Here we use PEAKS Protein ID as an example to demonstrate the exporting results.

For example, in order to export your PEAKS results file to Excel or HTML, right click on the results file that you wish to export and select "Export Excel".

The following window will appear:

Here we provide two types of exporting functions: 'complete protein list' without peptide details, or 'MCP compliant output'. When you select MCP compliant output, you can check the "Export only Marked Protein(s) and Corresponding Peptides(s)" if you are only interested in some proteins and previously marked them in the result table.



OK Cancel

Bro

Help

Export Excel R	esult Report		×
Select the T	ype of Results to Export		
Comple Comple	te Protein List (Peptide Details Omitted)		
MCP co	mpliant output		
Ex	oort only Marked Protein(s) and Corresponding Peptide(s)		
Select the E	xport Destination		
×ls File:		Browse	
		OK Cancel	Help

The output of "Complete Protein List" consists of two major sections, one is the representations table which display a representative protein for each cluster, and another is the whole protein table which lists all the clustered proteins.

The MCP compliant output contains the two tables described above, however, it also provides more information in the whole protein table. These additions include all of the supporting peptides and their coverage within the protein. False discovery rate (FDR) estimation is also displayed if you have run PEAKS Protein ID search with decoy database. We also provide the 'Single-Peptide Based Protein' results table which contains all the proteins with only one supporting peptide detected.

#### **15.4 EXPORTING STATISTICAL GRAPHS**

We provide exporting statistical graphs for de novo and PEAKS Protein ID which is useful in results validation and analysis.

#### 15.4.1 EXPORTING STATISTICAL GRAPHS ON DE NOVO RESULT

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

De novo ALC Score Charts	ALC threshold:	0.3
🔘 Peptide Score Chart	Score threshold:	0.3
Protein Score Chart	Score threshold:	0.3
False Positive Rate Chart		
age Options		
asic Options Advanced Options		
Web	Description	
🔘 Print	Smalle viewin	st images, suitable for g online

Currently PEAKS provides the de novo ALC score chart where the user can input the ALC score threshold and select the image quality for export. PEAKS 5.2 will export a broken line graph reflecting ALC distribution. The x-axis is the ALC score and the y-axis is the number of peptides. Each data point (x, y) in the graph means there are y peptides at score x.



#### 15.4.2 EXPORTING STATISTICAL GRAPHS ON PROTEIN ID RESULT

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":

itatistics Graph Type		
De novo ALC Score Chart	ALC threshold: 0.3	
Peptide Score Chart	Score threshold: 0.3	
Protein Score Chart	Score threshold: 0.3	
False Positive Rate Chart		
mage Options		
Basic Options Advanced Options		
Web	Description	
Print	Smallest images, suitable for	
	viewing online	

Below are examples of the "Protein Score Chart" and the "Peptide Score Chart", respectively:

In the protein score distribution chart each data point (x, y), x is the rank of the representative of each cluster according to its PEAKS Score and y is the corresponding PEAKS score.

In the protein score distribution chart, for each data point (x, y), x is the rank of a peptide according its PEAKS Score and y is the corresponding PEAKS score.

# Chapter 16

# **16 ADVANCED CONFIGURATION AND ENVIRONMENT PREFERENCES**

# **16.1 PEAKS Environment Preferences**

This section will describe the settings of the environmental preferences configuration 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:

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

#### **16.1.1 GENERAL PREFERENCES**

*Default Input File Directory*- Select the "Browse" button to change the directory that will appear when adding data to a project.

**Default Output Directory**- PEAKS outputs your results to C:\PEAKSStudio5.2\derbyServer by default. Select the "Browse" button to change this location. This directory will be used for all derbyServer data processing.

**Project** folder- PEAKS uses C:\PEAKSStudio5.2\derbyServer\serverDB as the default output folder for project files. Select the "Browse" button to change this location. This must be a subfolder of the Output Directory. If you wish to change the location

Preferences		(
neral	General	
GUI	Default Input File Directory	
RMI Connections	C:\Users\brian	
Derby Database Performance		Browse
rument	Default Output Directory	
rch Engine Settings	Output Directory:	Browse
Socarys	C:\PEAKS_Studio_5.2\.\derbyServer	
	Project folder:	Browse
	C:\PEAKS_Studio_5.2\.\derbyServer\serverDB	
	Temporary File Directory	
	C:\PEAK5_Studio_5.2\.\temp	
		Browse
	Default Configuration File Directory	
	C:\Users\brian	
		Browse
	Default Log File Location	
	C:\Users\brian\.peaks\PEAKSLOG_0.txt	
		Browse

where projects are saved you must also change the location of the Output directory.

*Temporary File Directory*- PEAKS uses C:\PeaksStudio5.2\temp as the default temporary file output directory. 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. You cannot change this file's location.

*Default Log File Location*- Your log file for PEAKS can be found at C:\Documents and Settings\bsi\.peaks\peaks.log by default. You cannot change this file's location.

#### 16.1.1.1 GUI

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

Choose "Show Only the Top Protein in Each Cluster" to remove subset protein identifications from the protein display.

General Gut Connections Derby Database Performance Instrument Search Engine In Settings	User Interface Protein View Show Only the Top Protein in Each Cluster	
	OK Cancel	Help

#### 16.1.1.2 RMI CONNECTIONS

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

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.

Preferences		X
🖃 General	RMI Connections	5
GUI	Server Host:	localhost
Derby Database     Performance	Server Port:	33003
Instrument     ■	Client Port:	31003
<ul> <li>Search Engine</li> <li>Ion Settings</li> </ul>	Worker Port:	35003
		OK Cancel Help

#### 16.1.1.3 DERBY DATABASE

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

#### **Derby Host**

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

#### Derby Server Start Memory

The amount of memory used to start derby server will also come up by default but can be changed if more memory is available. It is not recommended to change this from the default

Preferences	
🖃 General	Derby Settings
GUI	Derby Host: localhost Port: 15270
Derby Database     Performance	Derby Server Start Memory: 512
Instrument     Search Engine	Derby Jar Location
H Ion Settings     ■	.//lib
	OK Cancel Help

setting. If you would like to increase performance use the performance settings or the memory utility.

#### Derby Jar Location

The "Derby Jar Location" panel will list the location of the Derby Jar file by default. This is displayed to find its location. Its location cannot be changed.

#### 16.1.1.4 PERFORMANCE

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

#### **Computer Performance**

Select low if you have between 512 MB and 1 GB of RAM. Select Medium if you have between 1 GB and 2 GB of RAM. Select High if you have 2 GB or more RAM.

#### 3D View

PEAKS will display a 3D view with your quantification results. Check the "Show 3D View" box to enable this function. PEAKS 5.2 comes with the Java3D program to support the viewing of 3D images. If you don't require this feature, deselect it to increase performance.

Preferences	X	3	
General	Performance		
GUI RMI Connections Derby Database Performance	Computer Performance		
Instrument			
	Show 3D View		
Ion Settings     ■     Ion Settings     ■	Advanced Options		
	Client JRE Binary Folder: C:\PeaksStudio5.2\jre\bin Browse		
	Client JVM Heap Size (MB): 1024		
	Start Compute Node Separately		
	Computing Node JRE Binary Folder: C:\PeaksStudio5.2.20100430\jre64\bin Browse		
	Computing Node JVM Heap Size (MB): 5000		
OK Cancel Help			

#### Advanced Options

These options are present for users who want to take full advantage of their 64 bit Windows operating system.

- Start client separately: Select this option to load the Client JRE separately. Select browse to choose the location of the Client JRE Binary folder. It is recommended that the Client JVM be a 32 bit JRE. The default setting is the 32 bit JRE which comes with PEAKS.
- Start Compute Node Separately: Select this option to load the Compute Node separately. For this, you can choose a 64 bit JRE downloaded from Java Sun by choosing the browse button. Select the location of the JRE binary folder. With a 64 bit JRE you can then toggle the Heap Space to any amount within the maximum physical memory of the computer.

#### **16.1.2** INSTRUMENT PREFERENCES

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

#### 16.1.2.1 ABI (.WIFF)

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

#### Default .wiff raw file convertor location

Click "Browse" to tell PEAKS the location of the Default .wiff raw file converter. MSX is a commercial .wiff converter developed by Infochromics is available from BSI.



#### 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.

Select Survey Spectrum Centroiding if centroiding has been performed before loading the data into PEAKS. Select Product Spectrum Centroiding if centroiding has been performed on the Product spectrum before loading into PEAKS. This is important to insure PEAKS performs optimally.

#### 16.1.2.2 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:

#### Default compass file location

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

#### Raw file converter options

CompassXport by default will export raw data. If you attempt to load raw data and no spectra are displayed choose to export line spectra.

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.

#### 16.1.2.3 SHIMADZU AXIMA (.RUN)

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

Click "Browse" to tell PEAKS the location of the Shimadzu run2xml.exe file.

#### 16.1.2.4 VARIAN (.XMS)

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

Click "Browse" to tell PEAKS the location of the xmlrai.exe file.

#### 16.1.3 SEARCH ENGINE PREFERENCES

#### 16.1.3.1 MASCOT SETTINGS

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

Preferences	
ieneral Instrument	Bruker (.yep/baf, fid files)
- AB1(.wiff)	Default Compass File Location
😑 Bruker(.yep/baf, fid)	C:Program Files\Common Files\Bruker Daltonik\AJDA\Compass\Xport.exe
Shimadzu AX3MA(.rut Varian(.xms)	Browse
iearch Engine	Raw Ella Convertor Online
on Settings	Compassion will export @ Raw data () Line sport
	Bruker . Itd file may contain several files, do you want to merge them into one data set? $\bigcirc$ yes $$@$ no
	OK Cancel Help

N Preferences	×.
🗄 General	Shimadzu AXIMA (.run)
🖃 Instrument	
- ABI(wiff)	Shimadzu run2xml.exe File Location
🖃 Bruker(.yep/baf, fid)	
Shimadzu AXIMA(.ru	Browse
Search Engine	
Ion Settings	
<	
	OK Cancel Help

N Preferences	8	
General  Instrument  ABL(.wiff)  Bruker(.yep/baf, fid)  Shimadzu AXIMA(.rur  Varian(.xms)  Search Engine  Ion Settings  Image Instange  Image Instange  Image Instange  Image Instange Image Instange Image	Varian (JXMS) Default xmirai exe Location	
	OK Cancel Help	



In this window you can 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.

N Preferences

- Mascot Settings

- XTandem Settings

X!Tandem Settings

X!Tandem Server Settings

X!Tandem Local Settings

C:\PEAKS Studio 5.2\.\xtandem

Hostname (or IP address):

Local Search

OK Cancel

Launch Serve

🗄 General

Instrument
∃ Search Engine

Ion Settings

#### 16.1.3.2 X!TANDEM SETTINGS

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

Start by selecting 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.

As PEAKS provides a local copy of X!Tandem, upon

installation, a default path will appear in the Local Settings section. If you wish to use another license/location for X!Tandem, click the "Browse" button to tell PEAKS where to find the search engine.

#### 16.1.3.3 OMSSA SETTINGS

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

As PEAKS provides a local copy, upon installation, a default path will appear here. If you wish to use another license/location for OMSSA, click the "Browse" button to tell PEAKS where to find the desired path.

#### 16.1.3.4 SEQUEST SETTINGS

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

To use Sequest, click the "Browse" button to tell PEAKS where to find the search engine. 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".

#### 16.1.4 ION EDITOR PREFERENCES

N Preferences		23
General     Instrument	Omssa Settings	
Search Engine Mascot Settings Carlot Settings	Default Omssa Path: C:\PEAKS_Studio_5.2\omssa	Browse
Ion Settings	OK Cancel	Help

23

Test Connection

Browse ...

Apply

Help

N Preferences		2
it General it Instrument	Sequest Settings	
Search Engine     Mascot Settings     YTapdam Settings	Sequest Location:	Browse
Omssa Settings     Sequest Settings	Default Sequest Parameter File (.params):	Browse
Ion Settings     ■	Sequest Result Output Folder:	Browse
	OK Cancel	Help

N Preferences	
<ul> <li>General</li> <li>Instrument</li> <li>Search Engine</li> <li>Ion Settings</li> </ul>	Ion Settings
	Show Decimal Places: 2 📩
	OK Cancel Help
	101

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

#### 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:

To select an ion type to be viewed in the ion table, click on the ion type in the "Ion Type" list found near 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 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 the "Remove from list <=" button. The ion type will now appear in the "Ion types" list.

5			
gine	Choose the ion types and	I their charges then add them to the ion tab	le column list.
25	Ion Types		Ion Table Columns
mm	A+000         A+000           A+000         B           B+0400         B+0400           B+0400         C+000           C+000         C+0413           X+0400         X+0400           X+0400         X+0400	1 => Add with charge = Add with charge = Remove from lat c=	<ul> <li>Match 1</li> <li>Match 2</li> <li>Match 2</li></ul>

### **16.2 PEAKS CONFIGURATION**

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

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

#### 16.2.1 ENZYME CONFIGURATION

PEAKS can use almost any enzyme or combination of enzymes in your analysis. You can select built-in enzymes from the extensive list provided in PEAKS 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 de la second state of the second state is		
PTM	csuit-in> Semi Gu C (phosphate) //  kaller (semi Lys C)		A New Enzyme
Database	<built-in> Semi Pepsin (pH 1.3)</built-in>		Delete Enzyme
Instrument	<built-in> Semi Proteinase K</built-in>		
Parameters	<bult-in> Semi Trypsin</bult-in>		E
	<built-in> Trypsin (Built-In&gt; Trypsin with [DIP]</built-in>		-
	Enzyme Details		
	Engine Manager (2010)		
	Enzyme Name: Trypan		
	Cleave Sites (X = all amino acids)		
	after + RK	and not before 🔟 P	
	or after -	and before 👻	
	or after +	and before +	
	or after +	and before +	
	Allow up to one end of a peptide to di	sobey the cleavage rule	
			Add/Update

the information 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 will be grayed out.

#### Create a new enzyme

Click on the "New Enzyme" button to create a new enzyme.

Enzyme Details: This is how you specify where the custom 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 where the cleave sites are by choosing 'after' or 'not after' and 'before' or 'not before' to specify the range. There is also an option to "Allow up to one end of a peptide to disobey the cleavage rule".

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, refer to section <u>6.1.2 Enzyme Options</u> or <u>7.1.2 Enzyme Options</u>, respectively.

#### 16.2.2 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 Details" panel will be grayed out.

Enzyme	F TH LISC		]
<ul> <li>PTM</li> <li>Database</li> <li>Instrument</li> <li>Parameters</li> </ul>	<built-in> 4-hydroxynonenal (HN <built-in> Acetylation (K) <built-in> Acetylation (N-term) <built-in> Angliadain (N-term) <built-in> Applied Biosystems de. <built-in> Applied Biosystems (R <built-in> Applied Biosystems (R <built-in> Applied Biosystems (IR <built-in> Applied Biosystems (IR Show unimod)</built-in></built-in></built-in></built-in></built-in></built-in></built-in></built-in></built-in>	E) avable ICAT(TM) heavy avable ICAT(TM) light AQ(TM) 4plex (K) AQ(TM) 4plex (N)	Delete PTM
	PTM Details PTM name: Mass (Monoisotopic): Neutral Inss mass (Monoisotopic):	4-hvdroxvnonenal (HNE) 156.11504 0.0	
	Residues that can be modified:	СНК	Anywhere -
	Formula:	H(16) C(9) O(2)	
	Rule:		Add/Update

#### Create a new PTM

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

- PTM 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 looses as a result of the PTM.
- Neutral loss mass: the mass that the modified residue looses as a result of fragmentation in Daltons. For example, 28 would signify a loss of 28 Daltons.
- 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.
- Chemical formula: the chemical formula of the PTM. This should correspond to the mass listed above.
- 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.

Note: For information on defining new PTMs "on the fly" for PEAKS de novo or PEAKS Protein ID, refer to section <u>6.1.3 PTM Options</u> or <u>7.1.3 PTM Options</u>, respectively.

#### 16.2.3 DATABASE CONFIGURATION

In addition to de novo sequencing of peptides, PEAKS 5.2 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 specify taxonomy with certain databases.

From the "Configuration" window select "Database" from the left hand side to change your database configuration. The "Database List" at the top of the screen

,	Database List
	* Sample DB New Database
abase	Delete Database
rument	
Parameters	Set As Default
	Database Details
	FASTA format database: UniProtKB/Swiss-Prot  Validate Database
	Basic Options
	Path: C:(PEAKS_Studio_S.2/Data(SampleD6.rasta erowse or Download Database
	Advanced Options - Fasta Title Format
	Rule to parse accession/id from FASTA title:
	>(sp())/*((.)>*()
	Rule to parse description nom PASTA title:
	Accession/id UKL:
	nctp://www.unproc.org/unproc/
	Delimiter: [s+\(.*\)
	Taxonomy Options
	taxonid Browse Download
	taxdmp Browse Download
	Add/U

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.

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.

3) In the basics option panel, enter a name for your database and select "Download Database". A window will appear confirming the database chosen to be downloaded from the respective FTP or website.

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.

If you chose "Other" (in step 2) you must enter parsing parameters by completing the textboxes. Alternatively, if the database format is the same as one of the public databases such as NCBI-nr, you can choose to apply that database's parsing rules when PEAKS reads the database. To do this, simply select the database that is similar to yours from the drop-down 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 refer PEAKS where to find them in the "Taxonomy Options" panel.

11) To download the taxonID file, click the "Download" button. A window will appear confirming the ftp or website which has been identified as to the location of the desired database.

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. Be sure to save the file in a location that is accessible by PEAKS. Please note 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 color 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 "ncbinr.fas", database file or "ncbi\_nr.fas" rather than "ncbi nr.fas".

Enzyme PTM Database Instrument Parameters	Manufacturer:General FT-trap FT-trap (ecd-cid) FT-tran (etd)		New Instrument	
PTM Database Instrument Parameters	Manufacturer:General FT-trap FT-trap (ecd-cid) FT-tran (etd)		New Instrument	
Database Instrument Parameters	FT-trap FT-trap (ecd-cid) FT-trap (etd)			
Instrument Parameters	FT-trap FT-trap (ecd-cid) FT-trap (etd)			
Parameters	FT-trap (etd)		Delete Instrument	
r di dinocoro			E.	
	FT-trap (pqd)			
	FTMS			
	FTMS (ecd) FTMS (ecd-cid)			
			•	
	Instrument Details			
	Instrument Name: FT-tr	an		
	Basic Options	op		
	Manufacturer:	_General	-	
	Ion Source:	ESI(nano-spray)	Ŧ	
	MS Precursor Scan:	FT-ICR/Orbitrap	Ŧ	
	Fragmentation Type:	CID, CAD, IRMPD (y and b ions)	Ŧ	
	MSn Product Scan:	Linear Ion Trap	<b>T</b>	
	Advanced Options			
	Precursor mass search by	ne: Monojostopic 🖉 Average		
	Processor mass search cyp	Per O Honoboscopic O Hiterago		
	Parent mass error toleran	ice: 0.01	Da 👻	
	Fragment mass error toler	rance: 0.5	Da 👻	

#### 16.2.4 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: 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.

In the "Instrument Details" panel, create a name for your instrument.

Next, fill in your details in the "Basic Options" panel. In the manufacturer drop-down list, select a specific vendor or "General".

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.

MSn Product Scan: Use the drop-down list to select what type of MSn 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 were used. This will allow 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.

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

#### **16.2.5** PARAMETER CONFIGURATION

From the "Configuration" window select "Parameters" from the left hand side 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, SPIDER parameters or other parameter categories. 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

If you would like to create and save new parameters, you can do this during or before you set up auto de novo

Configuration	X
🖉 Enzyme	Parameter List
🖉 PTM	Parameter Type: De novo    Delete
🥙 Database	Instrument_default
Instrument	ITRAQ Sample
Parameters	Label Free Sample
	Parameter Details
	De Novo Predefined parameters Instrument_default   Save as
	Error Tolerance
	Parent ion: 0.01 Da Fragment ion: 0.5 Da
	Enzyme
	Trypsin   View Enzyme New Enzyme
	РТМ
	Set PTM
	Remove
	Switch tune
	Since (spo
	Maximum allowed variable PTM per peptide 3 💭
	General Options
	Preprocess this data on-the-fly (peak centroiding, charge deconvolution and deisotope)
	Report up to 5 🜩 candidates per spectrum
	OK Cancel Help

sequencing (refer to section <u>6.1 Setting up Auto de novo Sequencing Parameters</u>), PEAKS Protein ID (refer to section <u>7.1 Setting up Protein Identification Parameters</u>) or SPIDER (refer to section <u>9.1 Setting up SPIDER</u> Parameters). These references will provide you with an explanation of all of the parameters.

#### Deleting a previously saved parameter

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

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

### **17** 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, computer and biological scientists as well as valuable consultants and beta-testers.

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, PatternHunter software for all types of homology search sequence comparison and ZOOM software for next generation sequencing.

# Chapter 18

### **18 PEAKS® SOFTWARE LICENSE**

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