

Progenesis QI for proteomics User Guide

Analysis workflow guidelines for HDMse and MSe data

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Introduction

This user guide takes you through a complete analysis of 9 LC-MS runs with 3 groups (3 replicate runs per group) using the unique Progenesis QI for Proteomics workflow. It starts with LC-MS data loading then Alignment, followed by Peak Detection that creates a list of interesting peptide ions (peptides) which are explored within Peptide Ion Stats using multivariate statistical methods then onto Protein identity and Protein Stats.

To allow ease of use the tutorial is designed to start with the restoration of an Archived experiment where the data files have already been loaded. The document covers all the stages in the workflow, initially focusing on the Automatic Processing of the data then the use of the individual analysis stages.

If you are using your own data files please refer to Appendix 1 (page 76) then start at page 6.

How to use this document

You can print this user guide to help you work hands-on with the software. The complete user guide takes about 60 to 90 minutes. This means you can perform the first half focused on LC-MS run alignment and analysis then complete the second half of analysis exploring comparative differences and Protein identity at a convenient time.

If you experience any problems or require assistance, please contact us at support@nonlinear.com

How can I analyse my own runs using Progenesis QI for proteomics?

You can freely explore the quality of your LC-MS data using Data Import and then licence your own LC-MS runs using this evaluation copy of Progenesis QI for proteomics. Instructions on how to do this are included in a section at the end of the user guide document.

LC-MS Data used in this user guide

For the purposes of this data set the MS^E parameters were set to 250:125 instead of the default settings as defined in Appendix 1 (page 76). This was done to reduce the time taken to demo the data analysis.

Workflow approach to LC-MS run analysis

Progenesis QI for proteomics adopts an intuitive **Workflow** approach to performing comparative LC-MS data analysis. The following user guide describes the various stages of this workflow (see below) focusing mainly on the stages from Alignment to Report.

	Review		Experiment	Review Peak	Peptide Ion	Identify		Refine	Review	Protein	
Import Data	Alignment	Filtering	Design Setup	Picking	Statistics	Peptides	QC Metrics	Identifications	Proteins	Statistics	Report
		-	0	0	0	-	0	0	0	0	10
										1000	0.0



Waters THE SCIENCE OF WHAT'S POSSIBLE."

Restoring the Tutorial

Open Progenesis QI for proteomics and download the Compressed (.zip) Tutorial Archive file from the 'User guide and tutorial' link shown below, placing it in a **new folder** on your desktop. Before restoring the tutorial in the software **you must** first right click on the (.zip) file and extract it to the same folder.

Now restore the uncompressed tutorial archive file. To do this, first locate the **Progenesis QI.p Tutorial HDMSe.Progenesis QIP Archive** file using the **Open** button and press Open.

QIP Progenesis QI for proteomics		
File		
Experiments		nonlinear
•		A Waters Company
Perform analysis Combine analysed fractions Recent experiments	Search	New to Progenesis QI for proteomics? Here are some resources to help you
QP Open Experiment Q→ → Progenesis QLp v4 HDMSe		get started with Progenesis QI for proteomics: • <u>The Progenesis QI for proteomics</u>
Organize New folder	₩ ▼ 🗍 🔮	workflow User guide and tutorial data Frequently-asked guestions
	Name Date modif	Prequency-asked questions Quickly go to an ion map location Want to quickly validate your sample running by zooming to a known ion? Go To Location R
File name:	✓ Experiments and Archives (*.Prc ▼) ✓ Open ▼ Cancel	Jump to a specific m/z and RT using the <u>Go To Location tool</u> in the top-left corner of the ion maps.
Other experiments	4.0.6381.30896	Latest blog posts Just because it's natural doesn't mean it's safe Stay in the fast lane with Progenesis at 45M5 500. Indiananolis

This opens the 'Import from archive' dialog.

Select the **Create a new experiment** option and select the folder in which you placed the archive, using Browse.

OP Import Experiment from Archive						
	from archive kperiment from this archive, any changes to the experiment will on below, not back to the archive.					
Replace an existin	g experiment					
Experiment to repla	ace: Progenesis QLp Tutorial HDMSe 🔹					
Oreate a new expension	eriment					
Experiment name:	Progenesis QI.p Tutorial HDMSe					
Save to folder:	and Demo Suites\Progenesis QI.p Tutorial HDMSe Browse					
	Import					

Then press Import.

Loading: Progenesis QI.p Tutorial HDMSe					

Note: use the **Replace an existing experiment** option if you want to over-write an existing version of the tutorial.

Tip: at each stage in the software there are links to more information and help on the website.

Stage 1: Import Data and QC review of LC-MS data set

The tutorial will now open at the Import Data stage (see below).



Each data file appears as a 2D representation of the run.

Note: the **Experiment Properties** are available from the File menu. These were selected when the experiment was created (see Appendix 1, page 76). The tutorial data is profile data.

Tip: the **'Mask areas for peak picking'** facility, accessed by right clicking on the run thumbnail) allows you to examine and exclude areas (usually early and/or late in the LC dimension (Retention Time)) that appear excessively noisy due to capture of data during column regeneration. This is not required for this data set.

Note: use the **Remove this run** to remove run(s) from the current experiment.

A Mask areas for peak picking...
X Remove this run Del

OP Experiment Properties

Runs in this experiment: 9 Machine resolution: High resolution Peak processing: Profile data

Now start the automatic processing.

X

Close

.

Stage 2A: Automatic Processing of your data

The Automatic Processing of your data can be set up and started before the import of your data has been completed by clicking on **Start automatic processing.**

Note: for this tutorial the data has been imported already.

2	 Perform automatic processing Click the button below to start automatic processing. This will analyse your data as far as possible, before suggesting the next step. 					
		Start automatic processing				
	are importing.	can be started while runs				

Setup of processing steps in the Analysis Workflow, up to and including Identify Peptides, can be performed in the Automatic Processing wizard, these include:

- Select an alignment reference
- Automatic alignment of all runs to a reference run
- Automatic peak picking for peptide ion detection
- Define an Experiment design
- Perform peptide and protein identification (for MS^E data only, as DDA data will require external database searching)
- Choosing a Quantitation method to be applied for abundance analysis

In this tutorial example you have 9 HDMSe LC-MS runs, so the automatic identification of peptides is available as part of the automatic processing.

As the runs have already been imported, click **Start automatic processing** to setup the Automatic processing wizard.

OPP Start automatic processing
Select an alignment reference To compensate for drifts in retention time, all runs in the experiment must be aligned to a single reference run.
How do you want to choose your alignment reference?
Assess all runs in the experiment for suitability
Use the most suitable run from candidates that I select
🔘 Use this run:
▶ A_01
For information on choosing the alignment reference, and why you might want to select your own candidates, please see the online guidance.
select your own candidates, please see the <u>omine guidance</u> .
< Back Next > Cancel

Progenesis QI for proteomics provides three methods for choosing the alignment reference run:

1. Assess all runs in the experiment for suitability

This method compares every run in your experiment to every other run for similarity, then selects the run with the greatest similarity to all other runs as the alignment reference.

If you have no prior knowledge about which of your runs would make a good reference, then this choice will normally produce a good alignment reference for you. This method, however, can take a long time for a large number of runs.

2. Use the most suitable run from candidates that I select

This method asks you to make a selection of reference candidates; the automatic algorithm then chooses the best reference from this subset of runs.

This method is appropriate when you have some prior knowledge of your runs suitability as references:

i.e when all the candidate runs are pooled samples or when all the candidates are from a condition that displays the largest set of common peptide ions.

3. Use this run

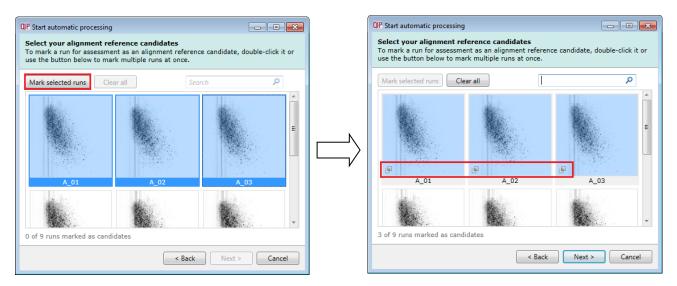
This method allows you to manually choose the reference run.

Manual selection gives you full control, but there are a couple of risks to note:

- If you choose a pending run which subsequently fails to load, alignment will not be performed.
- If you choose a run before it fully loads, and it turns out to have chromatography issues, alignment will be negatively affected (for this reason we recommend that you let your reference run fully load and assess its chromatography before loading further runs).

For this tutorial we will select the second option.

Select a subset of your runs as 'alignment reference candidates' and click **Mark selected runs.** An icon appears on the bottom left of each run to indicate that it is selected as a candidate.



On clicking **Next** you will be asked if you want to align your runs automatically.

The default is for automatic alignment, click **Next**.

QIP Start automatic processing	
Automatic alignment After selecting the experiment's alignment reference, the software can al- automatically align all runs.	50
After the alignment reference is chosen, do you want to start automatic align	ment?
< Back Next >	Cancel

The next page of the processing wizard will ask you if you want to **Perform peak picking** and allow you to set appropriate parameters.

QP Start automatic processing	- • •
Peak picking Peak picking is the process by which we locate the peptide ions and peaks in your samples.	their isotopic
After the automatic alignment is finished, do you want to start automat Perform peak picking	ic peak picking? Set parameters
< Back Next :	Cancel

For the purposes of this User guide we will use the default settings for peak picking except in the case of defining Retention Time limits where we will limit the peak picking to between 15 and 90 min.

Note: for more details on setting Peak Picking parameters refer to the section on Filtering (page 23)

Click on **Set parameters** and select the fourth tab to set **Retention time limits** for the detection. The default limits are displayed in the unticked boxes and detection will use these values whenever the box(s) are unticked.

QIP Peak Picking Parameters	•	Q	P Peak Picking Parameters		×
Runs for peak picking Peak picking limit Choose runs for peak picking — You can tick or un-tick each run to control which will be used by the peak picking algorithm. Although any run which is left un-ticked will not affect the peptide ion outlines, it will be available in the experiment design		Q	PP Peak Picking Parameters Runs for peak picking Peak picking lim Retention time limits You can set the minimum and maximum retention time for peak picking. Ions that elute before or after these values will be ignored.	Ignore ions before	Retention time limits
setup. Learn more about why you might not want to select all runs.	₩ C1 ♥ C2 ♥ C3				
	Start peak picking Cancel			Start pe	ak picking Cancel

Enter values of 15 and 90 min and tick the boxes as shown above.

Click **OK** to return to the Automatic Processing Wizard and click **Next** to Define an Experiment design.

QIP Start automatic pro	cessing	- • •
Experiment design Experiment designs experimental condit	allow you to group and compare	e your samples according to their
By defining an experi calculated automatic		al measures such as ANOVA can be
🔽 Set up an expe	iment design	
Enter a name fo	the experiment design:	
ABC		
Load the criteria	for grouping runs from this file:	
		Browse
Group runs by:	<no groups="" valid=""></no>	Ŧ
What file formats ar	e supported?	
	< Ba	ck Next > Cancel

To handle the grouping of your run data you can make use of **sample tracking information** that has been stored in a spread sheet at the time of sample collection and/or preparation.

Note: if a spreadsheet file of your data is not available you can create your experiment designs after the automatic analysis of the runs

For this example there is a **QIP_Conditions.spl** file available with the Experiment Archive you restored at the beginning of this tutorial exercise.

Give the experiment design a name (i.e A B C) and then use the **Browse** function to locate the **QIP_Conditions.spl** file.

OP Start automatic pro-	cessing	- • •			
Experiment design Experiment designs a experimental condition	allow you to group and compare your samples	according to their			
By defining an experin calculated automatica	nent design in advance, statistical measures such lly.	as ANOVA can be			
🔽 Set up an experi	ment design				
Enter a name for	the experiment design:				
ABC					
Load the criteria	for grouping runs from this file:				
10 Suites\Progen	esis QI.p_utorial for HDMSe_v3.0\QIP_Conditions	SPL Browse			
	VERSION	•			
	VERSION Condition				
	Location				
	User				
	Index				
What file formats are supported?					
	< Back Next	> Cancel			

To use this approach select the **Import design from file** option from the New Experiment Design dialog. Then locate the QIP_Conditions file and select what to **Group runs by**, for example: **Condition**.

Note: you can create additional experiment designs following the completion of automatic processing.

Select Conditions and then click Next.

For MS^E fragmentation data you can set up the peptide identification to be performed automatically.

Note: if the software has detected MS^E data then this option will be ticked by default.

QP Start automatic processing	OP Enter search parameters
Identify peptides Get identifications for the peptides in your samples using their MS ^t fragmentation data.	Enter the search parameters Select your FASTA file containing peptide and protein identifications: SWISSPROT-1.0 T Edit
After peak picking has completed, do you wish to automatically identify peptides? Use MS ⁴ data from my runs to identify peptides <u>Set parameters</u>	Enter the search parameters to use: Common search parameters Digest reagent: Trypsin Missed cleavages: Max protein mass: 250 Modifications: Carbamidomethyl C Nodifications: Search tolerance parameters Search tolerance parameters Search tolerance parameters Search tolerance parameters Change the digest reagents and modifications that are available for peptide searches. Modification editor Modification: Reagent editor Modification: Meagent editor Modification Reagent editor Modification editor Reagent editor Reagent editor
< Back Next > Cancel	Save parameters

Click on Set Parameters. The default Databank is for Swissprot-1.0 (which is a locked example).

To create a new Databank from a Fasta file click on **Edit** and then create a new one using the example FASTA file that is available with the Experiment Archive you restored at the beginning of this tutorial exercise (**Tutorial_nd_DB.fasta**).

For a new Databank click **Add.** Then give it name (i.e. Tutorial DB), select the parsing rules (UNIPROT) and specify the location of the FASTA file, see the example below.

QIP D	atabank Editor		
8	Name	Name:	Tutorial DB
8	SWISSPROT-1.0	Parsing rules:	
	Tutorial DB	-	
		Location:	C:\Users\andy.borthwick\Documents\Custo
	Add Remove		
			Save Cancel

The new Data bank will appear in the left panel now click **Save** to return to the Search parameters.

If your databank is not already displayed then select it from the drop down list.

Check the Common search parameters

The default settings are displayed:

Digest reagent: is set as Trypsin. Alternative Digest reagents are available from the list and additional ones can be added to the list using the **Reagent editor...**

Missed cleavages: is set as 1.

Maximum protein mass: is set at 250kDa

Modifications: are set Carbamidomethyl C (Fixed) and Oxidation M (Variable). More modifications are available from the list and additional ones can be added to the list using the **Modification editor...**

lon matching requirements: are set at

Fragments/peptide: 3, Fragments/protein: 7 and Peptides/protein: 1

Click Save parameters and then Next.

QIP Enter search param	eters	
Enter the search p Select your FASTA fi Tutorial DB	arameters le containing peptide and protein identific	cations:
Enter the search par	ameters to use	
Common search		
Digest reagent:	Trypsin	•
Missed cleavages:		1 max
Max protein mass:	250	kDa 🔹
Modifications:	Carbamidomethyl C	
	Add/remove modifications	
Search tolerance	e parameters	
 Ion matching 	requirements	
Fragments/peptide:	3	or more
Fragments/protein:	7	or more
Peptides/protein:	1	or more
Admin tools Change the digest re	eagents and modifications that are availa	ble for peptide searches.
	Modificatio	on editor Reagent editor
		Save parameters

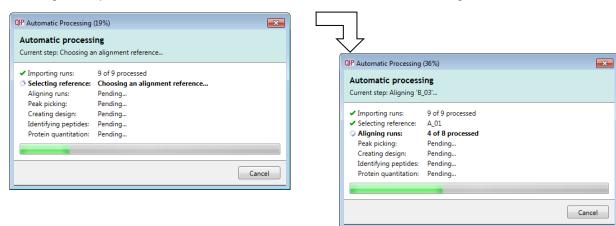
IP Start automatic processing	QIP Start automatic processing
Protein Quantitation If you've included a known amount of a calibrant protein in each of your samples, you can calculate the absolute amounts of each identified protein.	Protein Quantitation If you've included a known amount of a calibrant protein in each of your samples, you can calculate the absolute amounts of each identified protein.
Quantitation method:	Quantitation method:
Relative Quantitation using Hi-N	Relative Quantitation using Hi-N
Number of peptides to measure per protein (N):	Absolute Quantitation using Hi-N • Requires a calibrant protein to calculate absolute amounts • Uses mean calibrant abundance measured across runs • Uses the most abundant N peptides • Allows comparison between proteins within a run
	Relative Quantitation using Hi-N • Uses the most abundant N peptides • Allows comparison between proteins within a run
	Relative Quantitation using non-conflicting peptides Uses only peptides which have no conflicting protein identifications Allows comparison of a single protein across runs
Employ protein grouping, i.e. hide proteins whose peptides are a subset of another	Relative Quantitation using all peptides • Uses all peptides identified as part of a protein • Allows comparison of a single protein across runs
protein's.	Absolute Quantitation for HCP using Hi-N Requires a calibrant protein to calculate absolute amounts Uses calibrant abundance measured per run Uses the most abundant N peptides Calculates the amount of HCPs per run in fmol and ng

The **Protein Quantitation** dialog opens displaying the default method, **Relative Quantitation using Hi-N** which uses up to 3 peptides per protein to compute the relative amount of each 'identified' protein.

Use the drop down to reveal the alternative methods for protein quantitation

Select the Default option and click Finish.

The Alignment process starts with the automatic selection of A_01 as the alignment reference



Once Alignment completes Peak Picking commences followed by Design and Identification.

OIP Automatic Processing (44%) Automatic processing Current step: Analysing	
✓ Importing runs: 9 of 9 processed	QIP Automatic Processing (73%)
 ✓ Importing runs: ✓ Selecting reference: A 01 	Automatic processing
✓ Aligning runs: 8 of 8 processed	Current step: Searching A_02
Peak picking: Picking	
Creating design: Pending	✓ Importing runs: 9 of 9 processed
Identifying peptides: Pending	 Selecting reference: A_01
Protein quantitation: Pending	✓ Aligning runs: 8 of 8 processed
	 Peak picking: 46893 peptide ions found
	 Creating design: Created
Cancel	Identifying peptides: Searching
Cancel	Protein quantitation: Pending
	Cancel

Finally the Automatic Processing completes with the Quantitation being performed

OP Processing Complete	
Automatic process Time taken: 26 minutes 1	• •
 Importing runs: Selecting reference: Aligning runs: Peak picking: Creating design: Identifying peptides: Protein quantitation: 	
	<u>C</u> lose QC Metrics →

As the whole process proceeds you get information on what stage has been performed and also the % of the process that has been completed.

When Processing completes, depending on what stages you selected to perform, the Wizard displays what stage the workflow will open at. In this example it will open at QC Metrics. You can either:

- Continue with the analysis, as the Processing dialog is not displaying any warnings, and review QC Metrics. In which case you can go to page 52
- Open the analysis at QC Metrics and immediately return to the Review Alignment stage by clicking on it in the Workflow to review alignment quality (page 16)
- Alternatively you can **Close** the dialog. This will not move you to a later stage in the workflow but instead allow you to navigate through all the stages yourself or jump from stage to stage.

QIP Progenesis	QI.p Tutorial H	HDMSe - Proge	enesis QI for pro	teomics								
File Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Review Proteins	Protein Statistics	Report	A Waters Company
Import Dat	ta			A_01				< <	Actions 🔻	>>		Ø Help ▼

Note: if processing fails to complete successfully there are a number of suggested strategies you can use to proceed with your analysis. These are out lined in Appendix 2 (page 81)

Please Note: the time taken to automatically process this data through all the steps including Peptide Identification takes approximately 45 minutes (as shown on the previous page). This is dependent on the specification of your PC.

Note: this does not include the time to load the data

You can explore and re-perform the steps, sequentially and/or as part of the automatic processing as described in this guide.

For this dataset the alignment and detection from the automatic processing was of good enough quality to not require any further amendments. However, in the course of exploring the data you may choose to edit and re-perform the alignment and or the peak detection as described in this document.

You will be warned that you are about to lose the analysis as you 'unlock' a step and reanalyse.

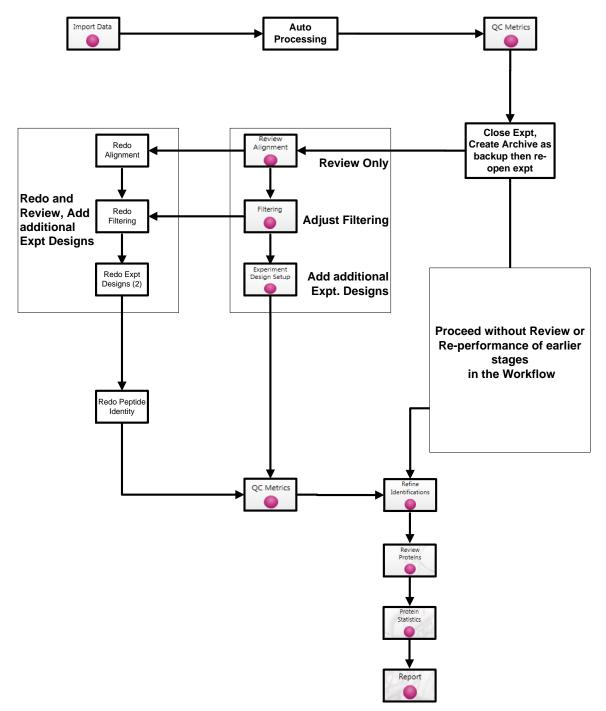
An example of the time it takes to perform each step is outlined in Appendix 10 (page 103).

The next stage in this document gives you a graphical view of how to proceed with your analysis following automatic processing.

Stage 2B: After Automatic Processing

When Processing completes, depending on what stages you selected to perform, the Automatic Processing Dialog displays (on the right) what stage the workflow will open at.

The flow chart gives you an overview of the various steps you can take to proceed with your automatically processed data.

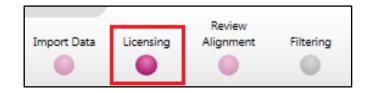


In this example, as the data is HDMSe (Waters) it is going to open at QC Metrics if you chose to perform all the steps in the Automatic Processing Wizard so you can either:

- Continue with the analysis, as the Processing dialog is not displaying any warnings, review the analysis at QC Metrics before proceeding with Refine Identifications. In which case you can go to page 52.
- If warnings are displayed or on Review of the Quality Metrics you require to review/redo earlier stages in the analysis then proceed to pages 16 and Appendices 1 (page 76) and 2 (page 81).

Stage 3: Licensing

This stage in the analysis workflow will **only** appear if you are using 'Unlicensed' data files to evaluate the software and have no dongle attached.



If you have performed an analysis using Automatic Processing without a valid dongle or do not have the appropriate code to licence your runs, if you close Progenesis QI for proteomics you will be warned that the analysis will be lost.

File port Data Licensing Alignment Filtering Design Se		A Waters Compa
ongle License Runs		
This installation is currently restricted to analyse icensed runs only.	Run name Licenc state	ce License this run
To license your runs, you need an evaluation or	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicent	nsed 🔽
ease licence code which can be obtained from	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicer	nsed 🔽
a sales representative.	$\label{eq:c:lustomer} C:\label{eq:c:lustomer} U = C:\label{eq:c:lustomer} C:\label{eq:c:lustomer} U = C:e$	nsed 🔽
Once licensed, your runs can be analysed on	$\label{eq:c:Users} C: Volume to Vo$	nsed 🔽
any installation of the software. The licence is	$\label{eq:c:lusers} C: \label{eq:c:lusers} $	nsed 🔽
automatically included when archiving an experiment.	$\label{eq:c:Users} C: Volume to Vo$	nsed 🔽
f your runs have been licensed on another	$\label{eq:c:Users} C: Volume to Vo$	nsed 🔽
computer, <u>click here</u> to make the licences	$\label{eq:c:lusers} C: \label{eq:c:lusers} C: \label{eq:cusers} C: \$	nsed 🔽
available on this computer.	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicer	nsed 🔽
f you have one, you can <u>open a licence file</u> to nstall.	QP Unable to save experiment	
f you have just installed a dongle, <u>click here</u> .	You cannot save the analysis without a valid license. If you close now your analysis will be lost.	
	OK Cancel	
	Run licence code: Use	Licence Code

Note: although the analysis will be lost (Alignment Peak Picking etc) the experiment file and all the 'loaded pre-processed runs are retained'. This allows you to reopen the experiment, when you have a licenced code and/or dongle available, and redo the analysis steps without having to reload the data.

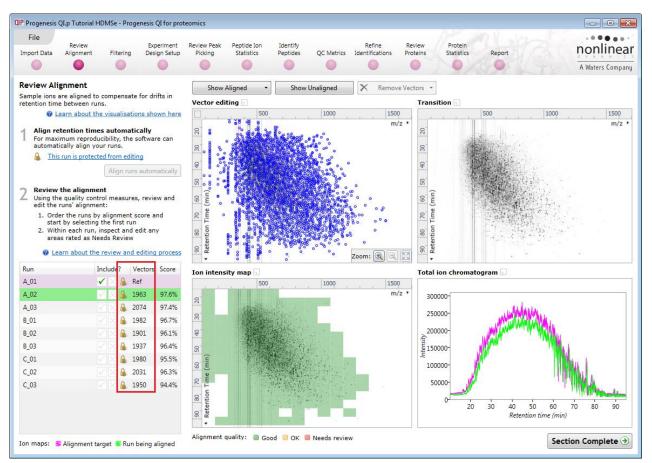
For more details on managing the licencing of your data refer to Appendix 3 (page 83)

If you are using the tutorial archive, this page will not appear as the data files are licensed.

Stage 4: Review Alignment

At this stage Progenesis QI for proteomics **Review Alignment** opens displaying the alignment of the runs to the Reference run (A_01).

Having performed the analysis automatically there will be icons next to each run in the vectors table indicating that the run is protected from editing.



In the course of reviewing the quality of alignment, you may decide that the alignment requires editing.

To do this click on the **This run is protected from editing** link above the 'greyed out' **Align runs automatically** button.

Review Alignment Sample ions are aligned to compensate for drifts in retention time between runs.	
Q Learn about the visualisations shown here	QP Protected from editing
Align retention times automatically For maximum reproducibility, the software can automatically align your runs. Image: This run is protected from editing Align runs automatically	Delete existing analysis? If you change the alignment, it will invalidate the current analysis including peptide ion pattern and IDs, editing, and tags. If you want to keep these, you should archive this experiment before changing the alignment and moving forward.
2 Review the alignment Using the quality control measures, review and edit the runs' alignment:	Delete analysis and allow editing Cancel
 Order the runs by alignment score and start by selecting the first run Within each run, inspect and edit any areas rated as Needs Review Learn about the review and editing process 	,,

Note: as you click on the link, you will be warned that you are discarding the current analysis (all steps beyond alignment).

Details on editing alignment are described in Appendix 4 (page 84)

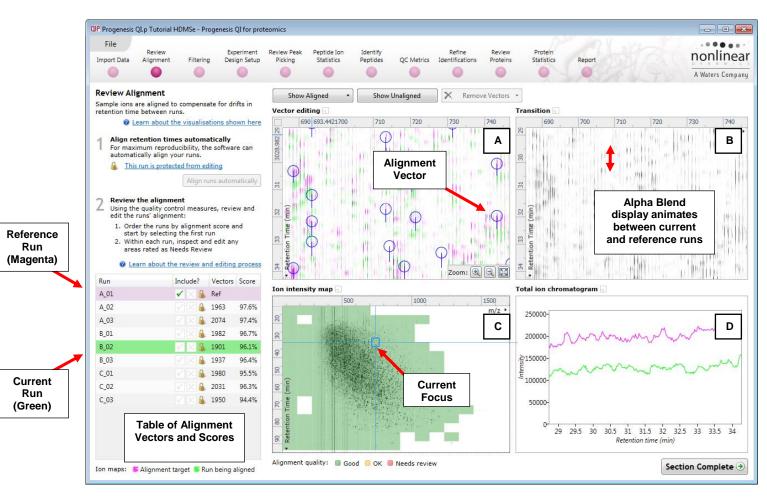
Layout of Alignment

To familiarize you with Progenesis QI for proteomics Alignment, this section describes the various views used in the alignment of the LC-MS runs

To setup the display so that it looks similar to the one below:

 In the Run table click on Run B_02 to make it current. You will now be looking at the alignment of B_02 to A_01 in the Unaligned view. Now drag out an area to review on the **Ion intensity map**. The other 3 views will update to reflect the new focus.

Run	Include?	Vectors	Score
A_01	🖌 🖂 🌡	Ref	
A_02	🗸 🗙 🌡	1963	97.6%
A_03	🗹 🗙 🌡	2074	97.4%
B_01	< X 🌡	1982	96.7%
B_02	$\mathbb{Z}\times\mathbb{A}$	1901	96.1%
B_03	$\checkmark \times \clubsuit$	1937	96.4%
C_01	\checkmark \times \clubsuit	1980	95.5%
C_02	$\checkmark \times \clubsuit$	2031	96.3%
C_03	$\times \times \clubsuit$	1950	94.4%



Vector Editing (Window A): is the main alignment area and displays the area defined by the current **focus** rectangle shown in Window C. The current run is displayed in green and the reference run is displayed in magenta. Here is where you can review in detail the vectors and also place the manual alignment vectors when required.

Transition (Window B): uses an **alpha blend** to animate between the current and reference runs. Before the runs are aligned, the peptide ions appear to move up and down. Once correctly aligned, they will appear to pulse. During the process of adding vectors, this view can be used to zoom in and also navigate thus helping with accurate placement of manual vectors.

Ion Intensity Map (Window C): shows the **focus** for the other windows. When you click on the view the blue rectangle will move to the selected area. The focus can be moved systematically across the view using the cursor keys. The focus area size can be altered by clicking and dragging out a new area with the mouse. This view also provides a visual quality metric for the Alignment of the runs, from green through yellow to red.

Total Ion Chromatogram (Window D): shows the current **total ion** chromatogram (green) overlaid on the Reference chromatogram (magenta). As the peptide ions are aligned in the **Vector Editing** view the chromatograms become aligned. The retention time range displayed is the vertical dimension of the Focus Grid currently displayed in the **Ion Intensity map** (Window C).

Reviewing quality of alignment vectors

After **Automatic alignment** the number of vectors and Quality Scores will be updated on the **Runs** panel and the vectors will appear (in blue) on the view.

Where the alignment has worked well, the alignment views will look as below with the Ion Intensity Map showing green indicating good quality alignment and the Transition view showing peptide ions pulsing slightly but not moving up and down.



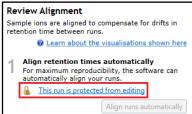
To simulate poor alignment following automatic alignment, place a single manual vector on the Vector editing view (Window A).

Placing an 'incorrect vector' to simulate miss alignment in Retention Time

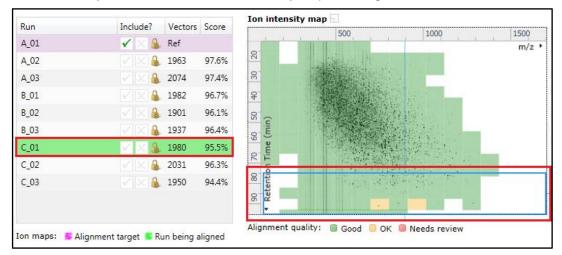
This section of the user guide is provided as a **demonstration of the alignment process**, it is **not** required for the normal analysis workflow of this data.

Note: if you do explore the alignment process the final steps in this section show you how to return the alignment to the state following automatic alignment

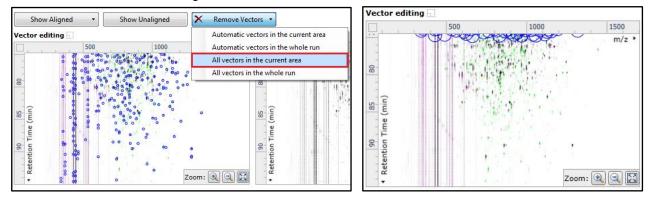
To edit the alignment of your data you must first unlock the analysis. To do this click on the link **This run is protected from editing** and then click **Delete analysis and allow editing**



First click on run **C_01** in the Runs table to make it current. Then remove the automatic vectors from 75 min onwards. To do this you must click on the lon intensity map and drag out an area as shown.



This resets all the other views. Now click **Remove Vectors** and select **All vectors in the current area** all the vectors in the Vector editing view will be removed.



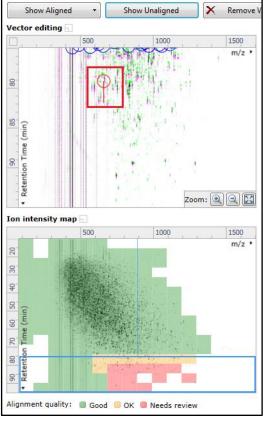
Now click on the Vector editing view and drag the view slightly upwards causing the two runs to go out of synch. Then release the mouse button.

By doing this a single manual vector will appear with a length corresponding to the 'drag'.

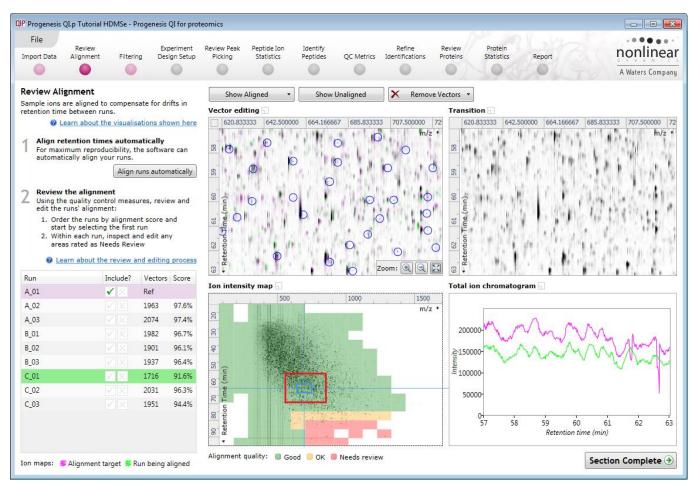
Note: the manual vector is **red**, to distinguish it from the automatic vectors (blue)

The effect of adding this incorrect manual vector is to reduce the Alignment score and also cause a significant proportion of the Alignment quality squares to turn red on the Ion Intensity Map.

Finally click on Show Aligned.



Using the **Simulated** miss-aligned example, you can explore the review process for alignment. The alignment looks as below with a region of poor alignment (highlighted in red).

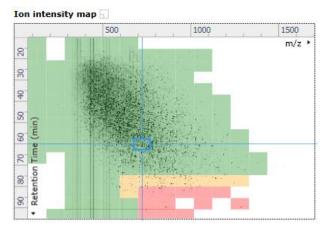


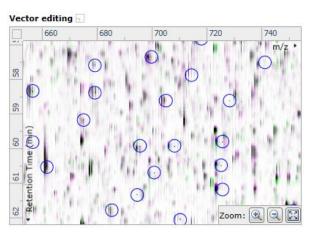
Note: now a smaller area in the 'green' well aligned region

Reviewing Quality of Alignment

At this point the quality metric, overlaid on the Ion Intensity Map as coloured squares, acts as a guide drawing your attention to areas of the alignment. These range from Good (Green) through OK (Yellow) to Needs review (Red). Drag out a 'Focus' area that corresponds to one of the coloured squares. Three example squares are examined here.

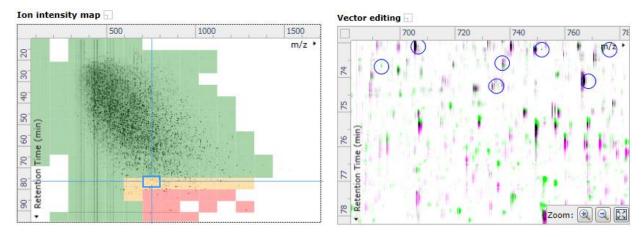
For a 'green' square, the majority of the data appears overlapped (black) indicating good alignment. When viewed in the Transition view the data appears to pulse.



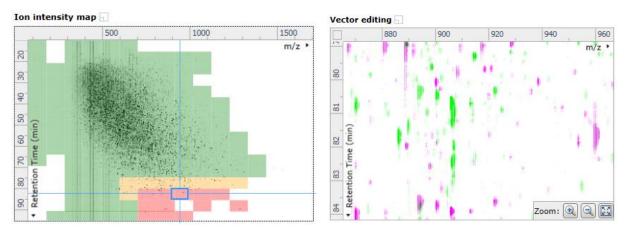


Waters

For a 'yellow' square some of the data appears overlapped (black) indicating OK alignment. When viewed in the Transition view some of the data appears to pulse.

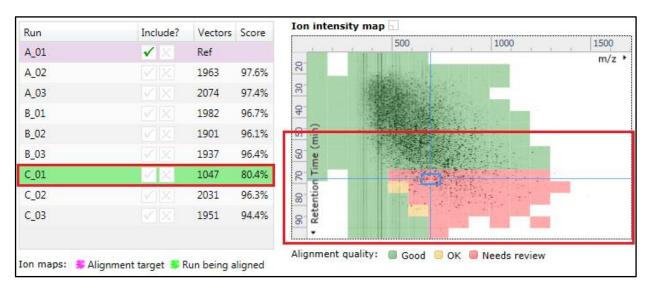


For a 'red' square little if any of the data appears overlapped (black) indicating questionable alignment. When viewed in the Transition view little data appears to pulse.



Note: the coloured metric **should be used as a guide**. In cases where there are a few 'isolated' red squares this can also be indicative of 'real' differences between the two runs being aligned and should be considered when examining the overall score and surrounding squares in the current alignment.

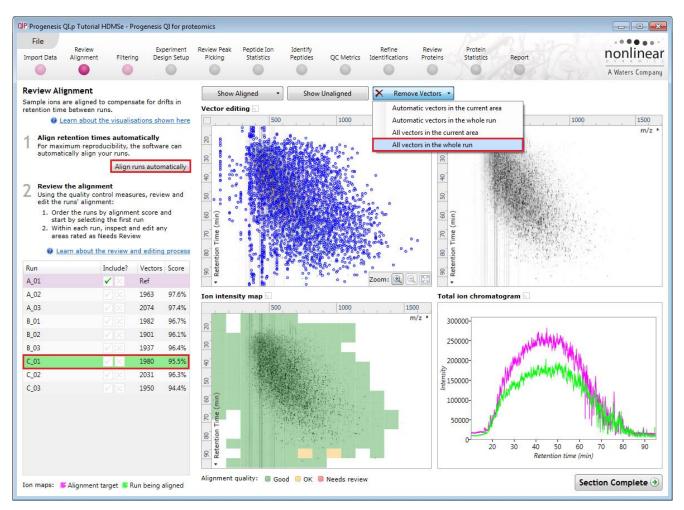
The weighted average of the individual squares gives the overall percentage score for each run's alignment.



Note: a marked red area combined with a low score clearly indicates a 'misalignment' and may require some manual intervention (see Appendix 4, page 84).

The alignment quality of this tutorial data set does not require any manual intervention so before going to the next section make sure you have removed all manual vectors and re-performed the Automatic alignment.

To do this for C_01 first select 'All vectors in the whole run' from the Remove Vectors and then click Align runs automatically.



Having aligned the runs automatically, click Section Complete to move to Filtering.

Stage 5A: Filtering

The Peak picking Parameters dialog opens if Picking has not been performed. If it has been performed, move to section 5B.

Peak Picking Parameters

The Peak Picking Parameters dialog opens, showing all the runs in the current experiment and a tick against each run. This is the default setting, where the peak picking algorithm uses information from all of the runs to contribute to the pattern of peptide ion outlines.

QP Peak Picking Parameters	•••	OP Peak Picking Parameters
Runs for peak picking Peak picking limi	ts Maximum charge Retention time limits	Runs for peak picking Peak picking limits Maximum charge Retention time limits
Choose runs for peak picking – You can tick or un-tick each run to control which will be used by the peak picking algorithm. Although any run which is left un-ticked will not affect the peptide ion outlines, it will still have outlines added to it and will be available in the experiment design setup. Learn more about why you might not want to select all runs.	 ✓ Run ✓ A_01 ✓ A_02 ✓ A_03 ✓ B_01 ✓ B_02 ✓ G_01 ✓ C_01 ✓ C 02 	Sensitivity You can adjust the sensitivity of the peak picking algorithm using these different methods. Each sensitivity method examines the intensities of groups of MS peaks to judge whether they are likely to form part of an ion or whether they represent noise and so should be ignored. Peaks that are rejected as noise will not be used to build ion outlines.
	C_02 C_03 Start peak picking Cancel	Chromatographic peak width The chromatographic peak width gives the length of time over which an ion has eluted. If you set a minimum peak width, any ion that has eluted over a shorter period will be rejected. Minimum width: 0 minutes Start peak picking Cancel

Tip: It may be appropriate **only** to pick peaks that are present in a limited number of your runs. In which case un-tick the runs that you do **NOT** want to contribute to the peptide ion detection pattern. This may be important when one or more of the runs appear noisy due to non-optimal chromatography or sample handling.

Note: peptide ions outlines will be added to 'un-ticked' runs; although these runs will not contribute to the peak picking pattern.

Tip: depending on run quality, a suggested minimum number of ticked runs should include at least one replicate of each experimental condition.

The sensitivity of the detection can be controlled by adjusting settings under the **Peak picking limits** tab.

Note: for all 3 Sensitivity modes a Chromatographic
peak width (Retention time window) for the peaks can be
set by applying a minimum retention window or peak
width in minutes.

Apply a minimum peak width						
Minimum width:	0.5	minutes				
		Apply a minimum peak widt				

For the runs in this user guide we will use the default settings for the Automatic method and NOT apply a minimum peak width.

The third tab allows you to set the **Maximum charge** of the ions, which will be detected. The default setting is a charge state of 20.

Peak Picking Paramete	rs		
Runs for peak picking	Peak picking limits	Maximum charge	Retention time limits
Maximum allowat You can set the maxin of ions to be detected charge greater than th be rejected.	ole charge num charge . Ions with a	Maximum ion charg	
		Charter	eak picking Cancel

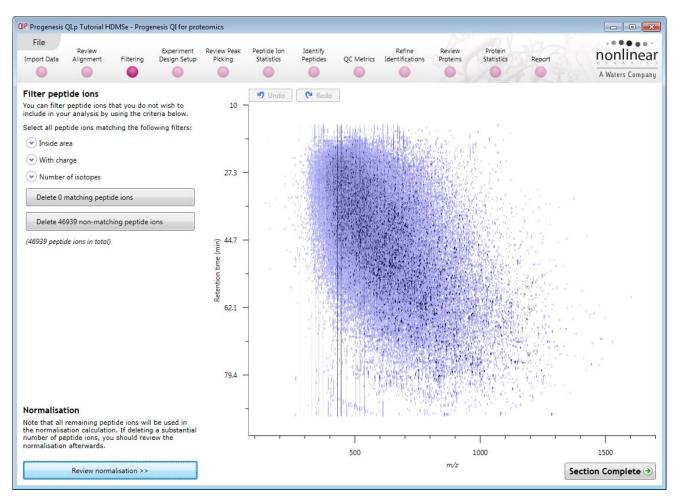
Finally, you can set **Retention time limits** for the detection. Current limits are displayed in the unticked boxes and detection will use these values whenever the box(s) are unticked.

QP Peak Picking Paramete	rs				×
Runs for peak picking	Peak picking limits	Maximum charge	Retention tim	ne limits	_
Retention time li	mits				
You can set the minim maximum retention ti picking. Ions that elut after these values will	me for peak e before or	I Ignore ions before	e 15	minutes	1
arter these values will	be ignored.	Ignore ions after	90	minutes	
	-				1
		Start pe	ak picking	Cancel	

For this dataset we will use the RT settings as shown above 15 and 90 minutes.

Press Start peak picking to start the detection process.

On completion of analysis, the Filtering stage will open displaying the number of peptide ions detected, in this example 46939.



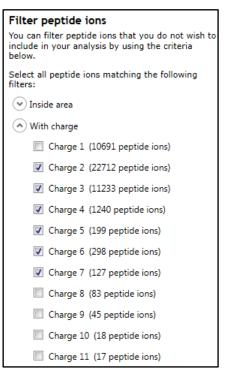
If required you can remove peptide ions based on position, charge state, number of isotopes or combinations of these peptide ion properties.

As an example, we will filter the peptide ions based on 'charge state'.

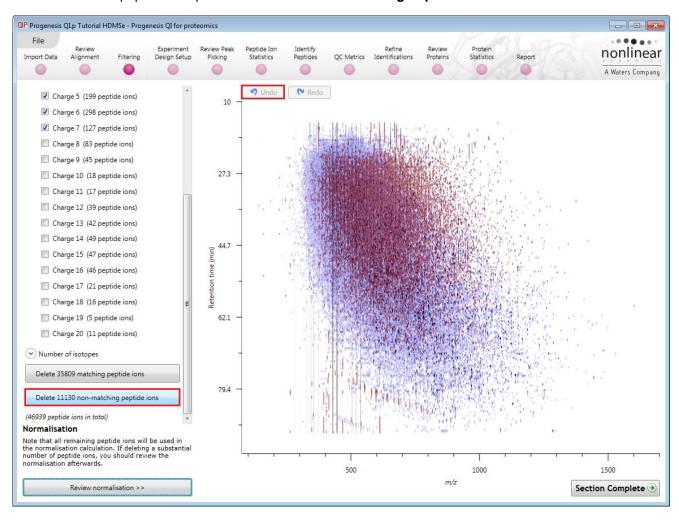
When **With charge** is selected the number of peptide ions present at each charge state is displayed, these can be selected accordingly. In this case we will retain peptide ions with a charge state of 2 to 7.

Tip: when filtering on one property of the peptide ions i.e. with charge, make sure you have 'collapsed' the other filters as expanded filters are applied concurrently.

Filter peptide ions	
You can filter peptide ions that you do include in your analysis by using the	
Select all peptide ions matching the fe	Filter peptide ions
inside area	You can filter peptide ions that you do not wish to include in your analysis by using the criteria below.
 With charge Number of isotopes 	Select all peptide ions matching the following filters:
	💌 Inside area
	With charge
	Charge 1 (10691 peptide ions)
	Charge 2 (22712 peptide ions)
	Charge 3 (11233 peptide ions)



Hence all peptide ions with a charge state of 1 or 8 and above will appear red on the main view as you hold the cursor over the appropriate delete button.



To remove these peptide ions press Delete 11130 Non Matching Peptide ions.

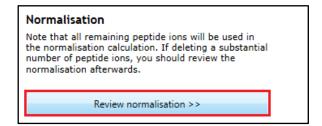
You can use the **Undo** button to bring back deleted peptide ions. For this tutorial we will proceed with the 35809 matching peptide ions.

Before moving on from filtering, you can review the normalisation of the experiment.

Tip: When you have reached the filtering stage, it is good practice to close the experiment and save an archive. This can be used to restore the unfiltered state if the filtering you have performed is too 'stringent'.

Stage 5B: Reviewing Normalisation

Normalisation review is accessed from the button at the bottom left corner of the filtering page.

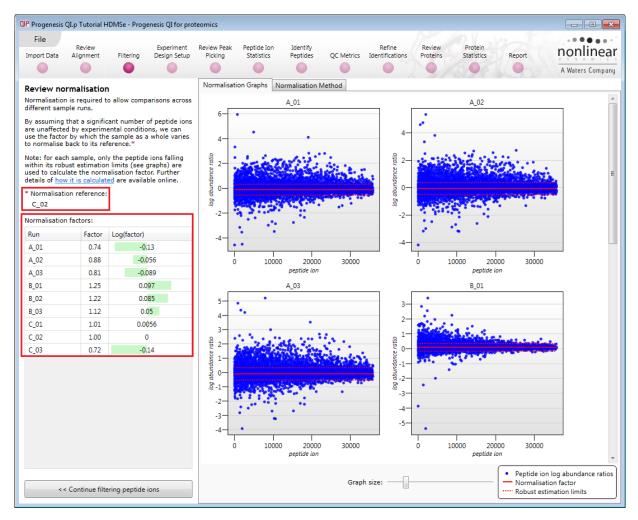


If you have filtered out a number of peptide ions from the original detection pattern then the normalisation will update.

Recalculating normalisation									

The **Review Normalisation** page will open displaying plots for the normalisation of all the peptide ions on each run.

This page in the workflow **does not** allow you to alter the Normalisation of your data but provides you with individual views for each run showing the data points used in the calculation of the normalisation factor for the run.



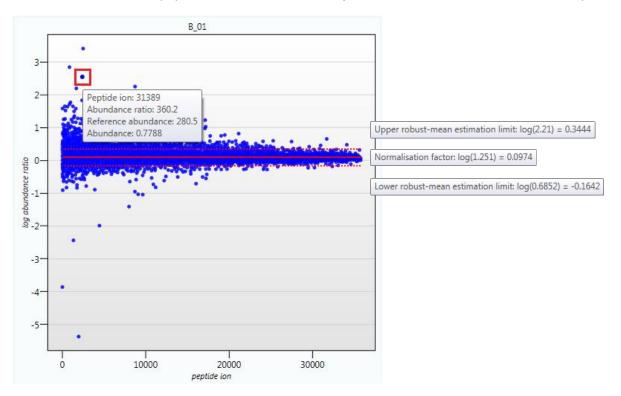
Normalisation factors are reported in the table to the left of the plots.

Calculation of Normalisation Factor:

Progenesis QI for proteomics will automatically select one of the runs that is 'least different' from all the other runs in the data and then set this to be the 'Normalising reference'. The run used, is shown above the table of Normalisation factors (in this example C_02).

For each sample run, each blue dot shows the log of the abundance ratio for a different peptide ion (normalisation target abundance/run abundance).

The details for individual peptide ions can be viewed as you hold the cursor over the dots on the plot.



On the graph the peptide ions are shown ordered by ascending mean abundance. The normalisation factor is then calculated by finding the mean of the log abundance ratios of the peptide ions that fall within the 'robust estimated limits' (dotted red lines). Peptide ions outside these limits are considered to be outliers and therefore will not affect the normalisation.

Finally, if you do **not** wish to work with normalised data then you can **use the raw abundances** by switching off the normalisation.

QP Progenesis	P Progenesis QLp Tutorial HDMSe - Progenesis QI for proteomics												
File Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Review Proteins	Protein Statistics	Report		A Waters Company
Review no	Review normalisation Normalisation Graphs Normalisation Method												
Normalisation is required to allow comparisons across different sample runs.			arisons across		o all proteins	·							
are unaffecte use the facto	different sample runs. By assuming that a significant number of peptide ions are unaffected by experimental conditions, we can use the factor by which the sample as a whole varies to normalise back to its reference. [*]			Normalise to	o all proteins o a set of house ny normalisatio		eins						

Note: once you have identified the peptide ions, you can then apply the **Normalise to a set of house keeping proteins** by using this option to locate and select the peptide ions.

For this experiment, you should leave the **Normalise to all proteins** option selected.

Now return to filtering by clicking on the button on the bottom left of the screen

	7-
<< Continue filtering peptide ions	

For this example, we **DO NOT** do any additional Filtering so click on **Section complete**.

Stage 6: Experiment Design Setup for Analysed Runs

At this stage in the workflow you can setup one or more experimental designs for your data.

There are two basic types of experimental designs:

Between-subject design: here samples from any given subject appear in only one condition. (i.e. control versus various drug treatments). The ANOVA calculation assumes that the conditions are independent and applies the statistical test that assumes the means of the conditions are equal.

Progenesis QI.p Tutorial HDMSe - Progenesis QI f	or proteomics									
File Review Expering Design:	etup Picking	Peptide Ion Statistics	Identify Peptides	0	Refine Identifications	Review Proteins	Protein Statistics	Report	<u>•</u>	Waters Comp
/hich experiment design type do y			reate	0-0 0-0 0-0	Vithin-subj u taken samp	-				
appear in only one condition? Then use the between-subject design.	Α		Delete Remove		under differe e the within-s			Before	During	After
To set up this design, you simply group the runs according to the condition (factor level) of the samples. The ANOVA calculation assumes that the		A2 [A2 Remove Note: you must have a sample from					x x1	X2	X3
conditions are independent and therefore gives a statistical test of whether the means of the conditions	c	C1 [Delete Remove Remove	For example, you would choose this type of design for a time series experiment where every subject has been sampled at each time point.		Detrient	Y Y1	YZ	Y3	
are all equal.	Add condition		<u>Remove</u>	To set up this design, you tell the			he	Z Z1	Z2	Z3
			I	A standa because assumpt repeated differen reduced conditio	ard ANOVA is n the data viola ion of indepen d measures AN ces can be elir as a source of n differences (more powerfu	tes the ANOV dence. With a OVA individua ninated or between (which helps t	A a Il			
				The with thought paired-s compari	nin-subject des of as an exten amples t-test t son between n d measures.	sign can be sion of the to include	1			

Within-subject design: here samples have been taken from a given subject under different conditions (i.e. the same subject has been sampled over a period of time or after one or more treatments). Here a standard ANOVA is not appropriate as the data violates the ANOVA assumption of independance. Therefore by using a *repeated measures* ANOVA, individual differences can be eliminated or reduced as a source of between condition differences. This within-subject design can be thought of as a extension of the paired samples t-test, including comparison between more than two repeated measures.

Additional information on how to apply the Within-subject Design is in Appendix 5 page 89

This experiment contains 2 conditions: Control and Treated and uses the **Between-subject design** to group the analysed runs to reflect the Biological conditions in the original study.

To create a new **Between-subject Design** hold the cursor over this option and click to open the dialog.

QIP Create New Experiment Design	—
Enter a name for the experiment design:	
ABC	(
How do you want to group the runs?	
Group the runs manually	
Copy an existing design:	Ŧ
Import criteria from a file:	Browse
Group runs by: <a> 	Ŧ
What file formats are supported? Create design	Cancel

Give the new experiment design a name and then click Create design.

QIP Progenesis QI.p Tutorial HDMSe - Progenesis QI	for proteomics			- • •
File Review Exper Import Data Alignment Filtering Design		des QC Metrics Identifications Proteins		Waters Company
ABCI × New				🕜 Help 🔻
Setup conditions	Runs Add Selected Runs to Cor	dition Search	D	
Setup the conditions that you want to compare (e.g., control, drug A, etc), and then assign eac your samples to the correct condition.			B_03	
A D	lete A			
A_01 Re A_02 Re	nove			
A_03 Re		C_02	C_03	
Add condition				
			[
			Section	Complete 🏵

To create a new condition

- 1. Select the runs for the condition by clicking on the required icon in the **Runs** panel, as shown.
- 2. Press the 'black triangle' next to the Add Selected Runs to Condition button on the main toolbar.
- 3. Select Add to new condition... from the drop down menu.
- 4. A new condition will appear in the **Conditions** panel on the left.
- 5. Rename the condition (e.g. C) by over typing the default name
- 6. Repeat steps 1 to 5 until all the runs are grouped into conditions.

An alternative way to handling the grouping of this set and other larger (and more complex) experimental designs is to make use of **sample tracking information** that has been stored in a spreadsheet at the time of sample collection and/or preparation.



For this example there is a **QIP_Conditions.spl** file available in the Experiment Archive you restored at the beginning of this tutorial exercise.

To use this approach select the **Import criteria from file** option from the New Experiment Design dialog. Then locate the QIP_Conditions file and select what to **Group by**, for example: **Condition**.

OP Create New Experiment Design	n	
Enter a name for the experime	ent design:	
AB		~
How do you want to group the	runs?	
Group the runs manually		
Copy an existing design:	A B C	Ŧ
Import criteria from a file:	ial_HDMSe\QIP_Conditions.SPL	Browse
Group runs by:	Condition	•
	VERSION	
What file formats are support	Condition Location	
	User	μ
	Index	

When Create design is pressed the new tab refreshes to allow you to adjust the conditions.

QIP Progenesis	QI.p Tutorial H	DMSe - Proge	nesis QI for prot	eomics								
File Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Review Proteins	Protein Statistics	Report	nonlinear
•	•	•	•	•	•	•	•	•			10-19	A Waters Company
ABC	ABI	× 🗋 Ne	w									🙆 Help 🔻
Setup con	ditions			Runs A	dd Selected Run	s to Condition	▼ Search	1	Q			
(e.g., control	nditions that y , drug A, etc), s to the correc	and then ass			C_01			C_02			C_03	
A			Delete		240			- 942)			-240).	
			A_01 <u>Remove</u>					- Starte			Contractor	
			A_02 <u>Remove</u>									
			A_03 <u>Remove</u>									
B			<u>Delete</u>									
			B_01 <u>Remove</u>									
			B_02 <u>Remove</u>									
			B_03 <u>Remove</u>									
Add condition	<u>on</u>											
											S	ection Complete 🏵

Use **Delete** on the Conditions panel to remove conditions that are not required in this particular design.

Note: On deleting a condition the runs will reappear in the Runs window.

Note: both designs are available as separate tabs.

To move to the next stage in the workflow, Review Peak Picking, click Section Complete.

Stage 7: Review Peak Picking and editing of results

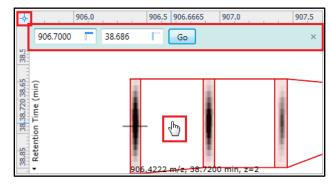
The purpose of this stage in the Workflow is to review the list of peptide ions using the visual tools provided and edit peptide ions if required.

The review stage has 5 display modes: 1D, 2D, 3D, Drift Time and Peptide ion Details controlled by the tabs on the bottom left of the display and the expander bar to the right of the table. Each display has multiple views to allow comparative exploration of the detected peptide ions on the aligned LC-MS runs.

Exploring analysed data using the Data displays

To set up the display as shown below, click on the Go To Location on the top right of the zoomed run view (right). Enter 906.700 m/z and 38.686 min and click **Go**. Then click on/or inside the peptide ion boundary to re-focus the view and table.

Window A: shows the list of peptide ions ranked by the p value for the one way **Anova** using the current Experiment Design (A B C).



Note: a value of 'Infinity' in the Fold column indicates 'Presence/Absence'



The 1D Display

Window B: displays the Mass spectrum for the current peptide ion on the selected Run (in window D). Hold the cursor over the peak to indicate m/z and intensity

Window C: displays the Chromatogram for the current peptide ion on the selected Run (in window D). Hold the cursor over the peak to indicate Retention time and intensity.

Window D: displays the details of the currently selected run. By default the selected run is an Aggregate view of all the aligned runs.

Details of individual runs can be viewed by using the 'Run' link and selecting the run you wish to view.

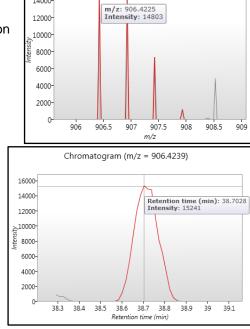
The peptide ion editing tools are located in this window (see page 41 for functional explanation).

Clicking on the Expression Profile tab in Window D shows the comparative behaviour of the peptide ion across the various biological groups based on group average normalised volume. The error bars show +/- 3 standard errors.

Window E: shows where the current peptide ion is located on the LC-MS run by means of the cross hairs.

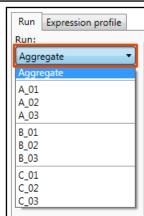
To change the current location, click on the image of the run (**note**: the retention time and m/z values update as you move the cursor around this view).

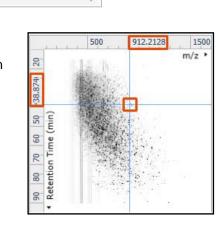
Note: doing this updates the focus of all the other windows.



Mass spectrum (38.72 min)

16000-14000-





Run Expression profile

ArcSinh Normalised Abundar

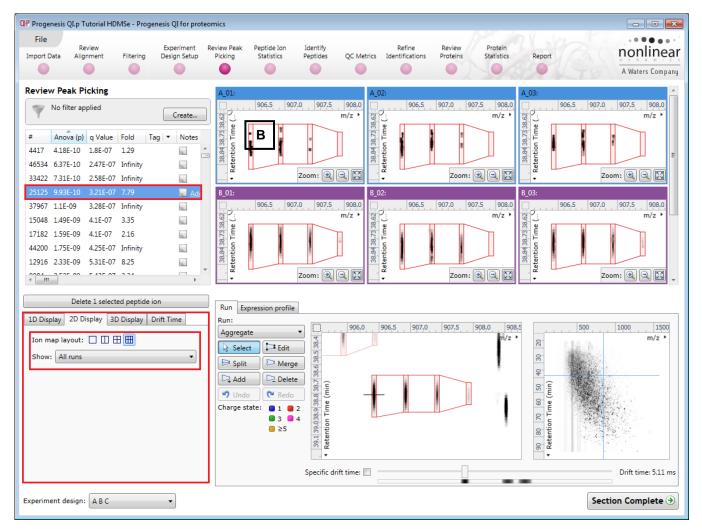
You can also drag out an area (blue square) on this view that will re-focus the other windows.



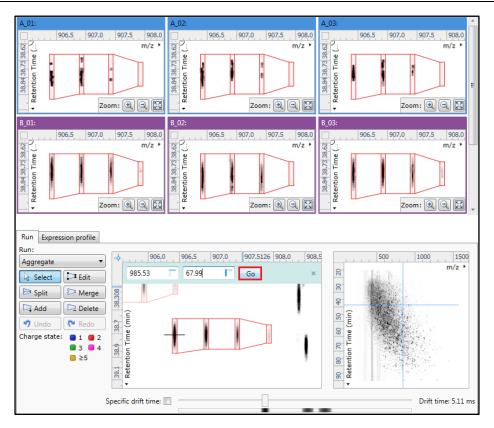
The 2D Display

Windows A, D and E: perform the same functions across all 4 display modes.

In the 2D Montage mode, Window B displays a montage of the current peptide ion across all the aligned LC-MS runs.



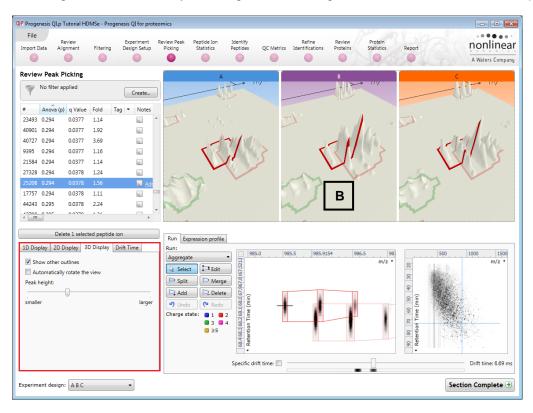
The appearance of the Montage (window B) is controlled by the panel on the bottom left of the display.



Using the the various views in the 2D display one can examine the peptide ion detection in detail to validate the correct detection of even fully overlapping peptide ions as shown below. (i.e Go To Location 985.53m/z and 67.99min)

The 3D Display

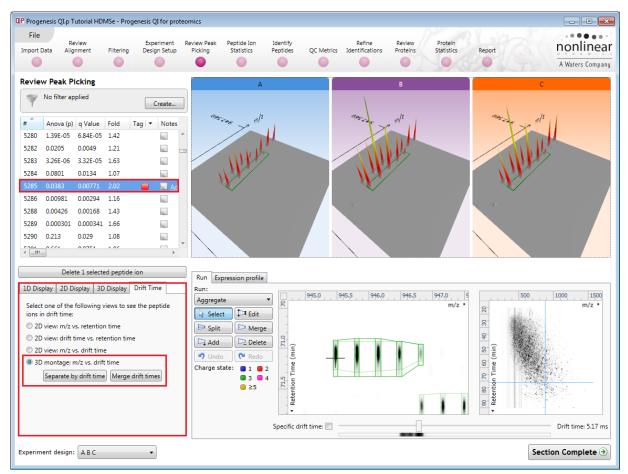
Window B changes into a 3D view by selecting the 3D Montage tab on the bottom left of the display.



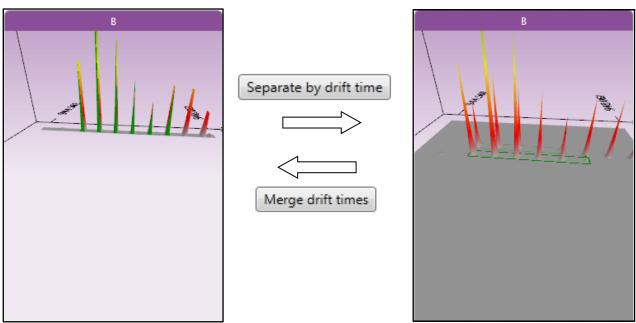
The views can be set to **Automatically Rotate** or you can rotate them manually by clicking and dragging them with the mouse.

Drift Time Display

When the Drift Time tab (\mathbf{F}) is selected in the bottom left of the display Window B changes into a 3D view displaying the Drift Time dimension (to examine this example order table on # and select 5285.



To view the drift time dimension for the current peptide ion, click on **Separate by drift time** on the Drift Time tab. This will expand the view showing the drift time separation between the detected peptide ions (below right)



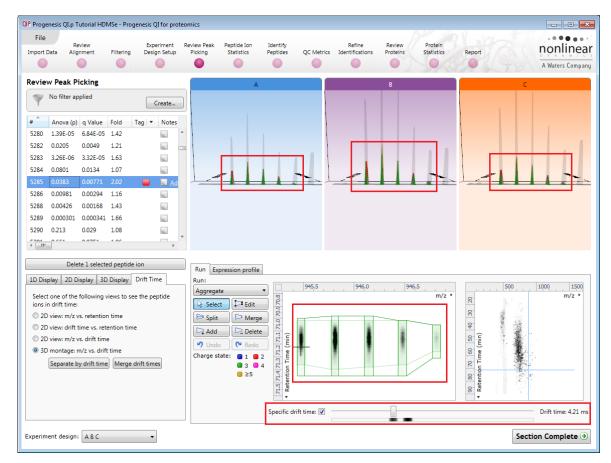
The left hand view displays the merged Drift Times.

Note: you can manipulate the orientation of the views by clicking on them and dragging the display to the required orientation. You can also zoom in and out of the panels by using the 'scroll' wheel on your mouse

Note: you can step through the specific **Drift Times** (measured in milli seconds) for the current peptide ion by clicking on the **Specific drift time** tick box at the bottom of the display.

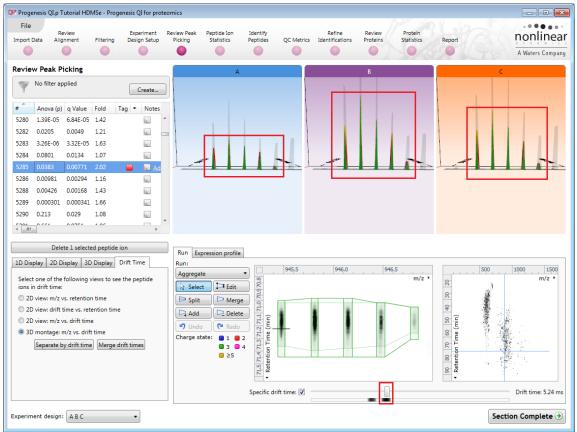


The 'crosshairs' on the peptide ion in the Run view identifies it as the current peptide ion in the table.



Waters

As you move the slider over the intense areas, indicated below, all the views update to the corresponding drift time.



Note: the crosshairs will remain on the original peptide ion in the table as you explore the Specific Drift times When you un-tick the 'Specific drift time, tick box the 3D views will return to showing the Merged Views for the current peptide ion in the table.



Using Quick Tags to locate examples of Drift Time

In the previous section, describing how to view Drift Time, you may have noticed the presence of a red 'Tag' in the table next to the peptide ion that we examined. Progenesis QI for proteomics allows you to assign tags based on the properties of detected peptide ions either through the manual sorting of the table or making use of the 'Quick Tags'. These tags can be used to filter the list of displayed data in order to aid exploration of the data.

To create a Quick tag for all peptide ions demonstrating separation by Drift time, right click on the table. Select **Quick Tags** then **Separated by drift time.**

In the new tag dialog either accept or overtype the tag name.

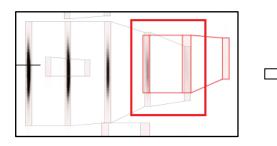
Create new tag		—
Separated by drift time	ОК	Cancel

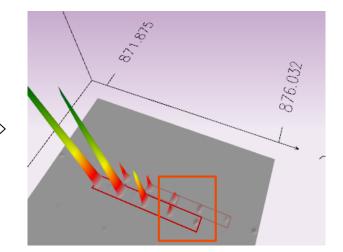
Review Peak Picking									А
Y	No filter ap	plied			Create				
#	Anova (p)	q Value	Fold	Tag	▼ Notes				
5284	0.0801	0.0134	1.07		Na.	*			
5285 5286	🦲 Sepa	rated by dri	ft time			_			
5288	New	tag			10.				
5289	Quic	k Tags		•	Anova	p-v	alue		
5290	🚰 Edit t	ags					hange		
5291	0.661	0.0751	1.06		Modifi No MS				
5293	0.00344	0.00146	1.17		No pro	otein	n ID		
5294	0.00127	0.000796	1.2		Separa	ted	by drift time		
5295	5.76E-06	4.33E-05	2.55		Identif	ied a	and separated b	oy drift	time

When the tag is created it will appear against those peptide ions that meet the criteria for the creation of the tag, in this case:

It tags peptide ions that overlap in both m/z and retention time but do not show an overlap in the drift time dimension i.e. those peptide ions that drift time has separated

For example the peptide ions below is overlapping at the same m/z and RT but are separated in drift time





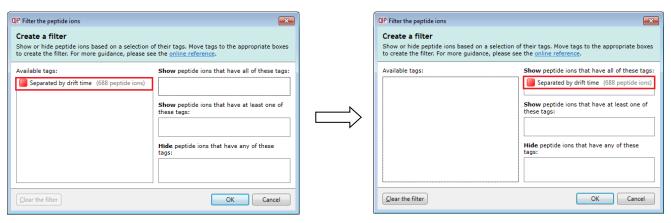
Now filter the table so that it currently only displays a list of peptide ions containing the **separated by drift time** tag.

Click on **Create** on the filter panel above the table.

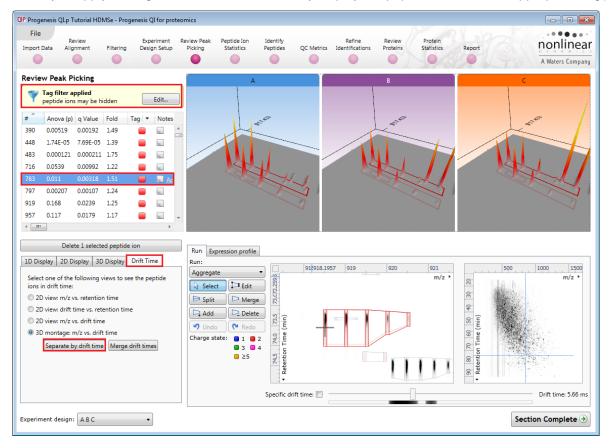
Vo filter applied	Y	No filter applied	Create
-------------------	---	-------------------	--------

This will open a Tag filter dialog, in this example, displaying that you have created assigned the **Separated by drift time** tag to 683 peptide ions in your experiment.

To display only those peptide ions containing this tag drag the **Separated by drift time** tag on to the **Show** panel and click OK.



When you apply the tag filter the table will now only display the peptide ions with the appropriate tag(s).



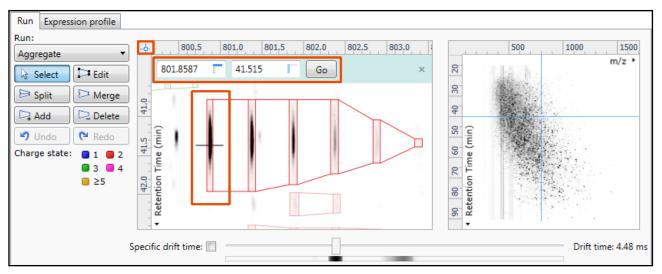
Note: with this **Tag filter applied** you can easily review the effect of Drift time separation for the peptide ions.

To remove the filter click on Edit, above the table, and Clear the filter followed by OK.

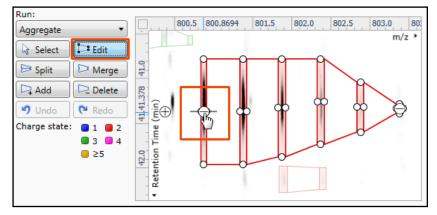
Editing of peptide ions in the View Results stage

As an example of using the editing tools which are located on the left of the LC-MS Run view, we will remove and add back the 'monoisotopic peak' for the detected peptide ion selected below. A peptide ion can be selected from the 'Peptide ions' list or located using the various views.

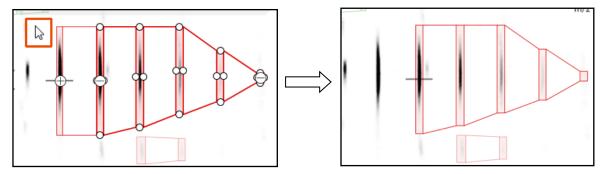
1. Locate the peptide ion at approx 801.86 m/z and 41.5 min using the **Go To Location** tool (top left of zoomed ion map), right click and zoom out and click the cross hairs on the monoisotopic peak to set the zoom.



2. Select the **Edit** tool and click on the peptide ion (in the Run view) to reveal the 'edit handles'. You can zoom in more by dragging an area around the peptide ion of interest.

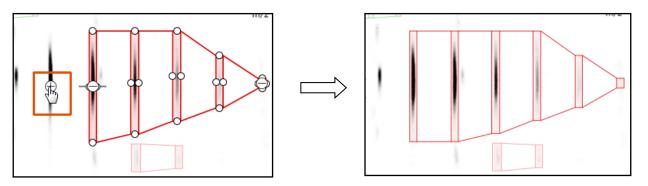


3. Click on the 'minus' handle over the monoisotopic peak to remove it.

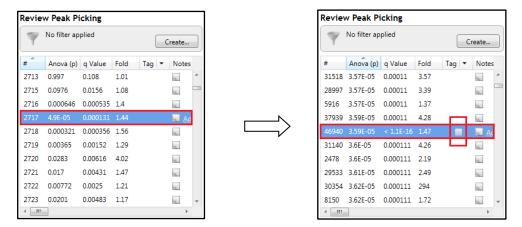


- 4. Click outside the boundary of the peptide ion to update the view.
- 5. To add a peak to an existing peptide ion, ensure that **Edit** is selected then click inside the peptide ion to reveal the handles.

6. Click on the 'plus' handle on the peak to add it.

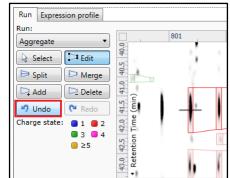


- 7. Then click outside the peptide ion to update the view.
- 8. Note: If you are not satisfied with the editing use the Undo button and retry.
- 9. **Note**: that a tag is automatically added to the edited peptide ion in the table and the peptide ions id. number is changed to the next available one at the end of the list.



The other tools: **split**, **merge**, **add and delete** behave in a similar fashion and their use can be combined to achieve the desired results.

Note: if you have been exploring the editing of peptide ions then a quick way of reversing the edit is to repeatedly press **Undo**.



Edit Tags	
🗄 🞦 New tag 🛛 al̪e Rename tag	🗙 Delete tag 🐒 Merge tags
Tag name	
Separated by drift time	Delete tag
Edited	
	OK Cancel
	Cancer

Then right click on the table and select **Edit tags**. Then select the automatically added **Edited** tag and press Delete tag.

Selecting and tagging peptide ions for Peptide Ion Statistics

There are a number of ways to 'refine' your 'Ranked List' of analysed peptide ions before examining them with the Statistical tools in **Peptide Ion Statistics**. These make use of simple 'Selection' and 'Tagging' tools that can be applied to the various groupings created in Stage 6 (page 29). An example is described below.

First expand the 'Peptide ions' table to show all the details by double clicking on the 'Splitter Control' to the right of the Review Peptide ions table.

Review	Review Peak Picking									
Y	No filter ap	plied	Create							
#	Anova (p)	q Value	Fold	Tag 🔻 Not						
18180	5.19E-05	0.000137	1.35	A						
21698	5.2E-05	0.000137	1.54							
35011	5.2E-05	0.000137	6.99							
28122	5.2E-05	0.000137	2.58							
2716	5.21E-05	0.000137	1.44							
10738	5.21E-05	0.000137	1.59	13.						
26113	5.22E-05	0.000137	10.2	a						
15956	5.22E-05	0.000137	1.91	10.						
12382	5.24E-05	0.000137	2.98	10.						
34456	5.24E-05	0.000137	2.68	· ·						
< _III_										

Then order on Abundance and select all peptide ions with an Abundance $> 2x10^4$.

Right click on the highlighted peptide ions and select 'New Tag '.Give the Tag a name. i.e. 'Most Abundant'.

File		eview Inment	Filtering	Experiment Design Setu		Peptide Ion Statistics	Identify Peptides	QC Metrics		Refine htifications	Review Proteins	Protein Statistics	Report		<u>0</u>	Waters Compa
eview	/ Peak P	icking														
Y	No filter ap	plied														Create
	Anova (p)	q Value	Fold	Tag 💌 N	otes	Highest Mean	Lowest Mean	m/z	z	Mass	RT (mins)	RT window (mins)	Abundance	Intensity	Max CV	(%) MS/MS
				-	Add a note											0 4
				5	Add a note											0
				5	Add a note											0
				Sepa	- irated by drift tim	e										0
										1041.616						0
332	0.0159	0.0041	1.26		tag :k Tags		С	369.6911	2	737.368	52.018	0.978	2E+04	4.16E+04	11.2	0
2250	0.00612	0.00214	1.21	_			С	451.7306	2	901.447	26.856	0.371	2E+04	1.85E+04	7.06	0
511	0.000199	0.000275	1.33	Edit :	tags		Α	478.2701	2	954.526	43.915	0.577	2E+04	4.24E+04	4.93	0
200	0.0162	0.00416	1.19	10	1	A	С	490.2539	2	978.493	40.783	0.749	2E+04	4.11E+04	7.75	0
893	0.00845	0.00265	1.56	10.		В	A	484.7865	2	967.558	27.913	0.354	2E+04	6.07E+04	17.5	ο.
D Disp		4361 select		ions Vrift Time	Run Ex Run: Aggrega Select Select Split Charge st	t Edit Merge Delete Redo	e (min)		78	83.0 783.0	5 784.0	794.5 795.0 m/z •	90 80 70 60 50 40 20 20 40 20 40 40 20 40 40 40 40 40 40 40 40 40 40 40 40 40	500	100	0 1500 m/z •
							Specific drift	time: 🔲 📒							D	rift time: 4.00 m

Note: there is already a red tag present that was assigned to those peptide ions that are **Separated by drift time**, which you created in the previous section.



	Review Peak Picking									
#	Anova (p)	q Value	Fold	Tag 💌	Notes					
7083	0.000313	0.00035		-	Ad					
					Ad					
				-	Ad					
					Ad					
				-	Ac					
5332	0.0159	0.0041	1.26		ha.					
12250	0.00612	0.00214	1.21		10					
4511	0.000199	0.000275	1.33		la.					
7200	0.0162	0.00416	1.19		la.					
2893	0.00845	0.00265	1.56		10					
٠										
	Delete	4361 select	ed pepti	de ions						

On clicking **OK** the Tag is added to the peptide ions highlighted in the table (signified by a coloured square, green in this example).

Now right click on any peptide ion in the table and select **Quick Tags** this will offer you a number of standard tag options. Select **Anova p-value....** Then set the threshold as required and adjust default name as required and click **Create Tag**.

Y	No filter a	pplied						
#	Anova (p) q Value	Fold	Tag 💌	No	tes	Highest Mean	Lowest Mean
7083	0.000313	0.00035	1.13		La.		В	А
6722	6.8	Separated b	v drift tin	me		Add a note	А	С
9223	0.0	Most Abund	-		4		В	С
2977	0.0	New tag			la.		В	Α
6561	5.6	Quick Tags		•		Anova p-value.		
5332	0.0	Edit tags				Max fold chang	e	
12250	0.0					Modification		
4511	0.000199	0.000275	1.33			No MS/MS data		I
7200	0.0162	0.00416	1.19			No protein ID		
2893	0.00845	0.00265	1.56			Separated by dr	ift time	
5782	0.00013	0.000219	1.5			Identified and s	eparated by drif	t time

QP New Quick Tag	-X
Where a peptide ion has:	
Anova p-value: ≤ ▼ 0.05	
Apply the following tag:	
Anova p-value ≤ 0.05	
Create tag	Cancel

Once this tag appears against peptide ions in the table right click on the table again and create another Quick Tag, this time for peptide ions with a **Max fold change** ≥ 2

OP New Quick Tag
Where a peptide ion has: Max fold change: 2
Apply the following tag: Max fold change ≥ 2
Create tag Cancel

The table now displays peptide ions with multiple tags.

The tags can be used to quickly focus the table on those peptide ions that display similar properties.

-	No filter applied	
T.		Create

Y	No filter ap	Cre	ate		
#	Anova (p)	q Value	Fold	Tag 💌	Not
14832	9.5E-05	0.000184	1.56		A
21897	0.449	0.0539	1.13		12
23412	0.0138	0.00372	1.19		la.
28296	0.0499	0.00937	4.1	-	la.
22593	0.0356	0.00731	1.2		la.
31637	0.0581	0.0105	1.21		
11500	0.00134	0.000816	1.18		12
13657	0.000774	0.000596	2.12	-	
30827	0.00098	0.000683	7.23	-	la.
25857	0.0139	0.00375	1.47		10
21893	0.188	0.0262	1.15		la.
19306	7.96E-05	0.000169	1.22		· ·

For example: to focus the table on displaying those peptide ions that are **Most Abundant** click on **Create** on the filter panel above the table.

Drag the tag on to the panel Show peptide ions that have all of these tags and press OK.

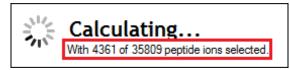
P Filter the peptide ions	×	QP Filter the peptide ions	
Create a filter show or hide peptide ions based on a selection woxes to create the filter. For more guidance, ple		Create a filter Show or hide peptide ions based on a selection boxes to create the filter. For more guidance, pl	
Available tags: Most Abundant (4361 peptide ions) Anova p-value ≤ Cu05 (20641 peptide ions) Max fold change ≥ 2 (7890 peptide ions) Separated by drift time (688 peptide ions)	Show peptide ions that have all of these tags: Show peptide ions that have at least one of these tags: Hide peptide ions that have any of these tags:	Available tags: Available tags: Max fold change 2 (7890 peptide ions) Separated by drift time (688 peptide ions)	Show peptide ions that have all of the tags: Most Abundant (4361 peptide ion Show peptide ions that have at least these tags: Hilde peptide ions that have any of the tags:
Clear the filter	OK Cancel	Clear the filter	ОК С

To move to the next stage in the workflow, Peptide Ion Statistics, click Section Complete.

Stage 8: Peptide Ion Statistics on Selected Peptide ions

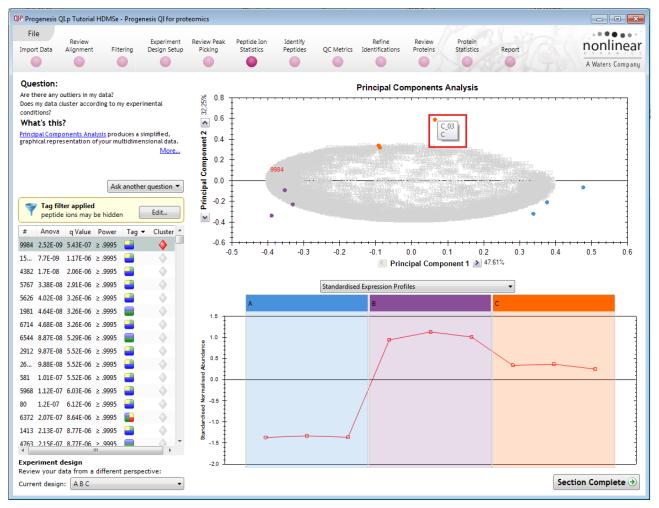
The user guide now describes the functionality of the Multivariate Statistics.

Peptide Ion Statistics opens calculating the Principal Components Analysis (PCA) for the active 'tag' in this case the **Most Abundant** peptide ions.



As an example we will start by examining the behaviour of the **Most Abundant** peptide ions from the previous stage, **Review Peak Picking**.

The statistical analysis of the selected data is presented to you in the form of interactive graphical representation of answers to questions asked of the analysed data.



Note: the LC-MS runs (samples) are displayed as solid coloured circles on the plot. To identify the runs, a tooltip is displayed when the cursor is held over each circle.

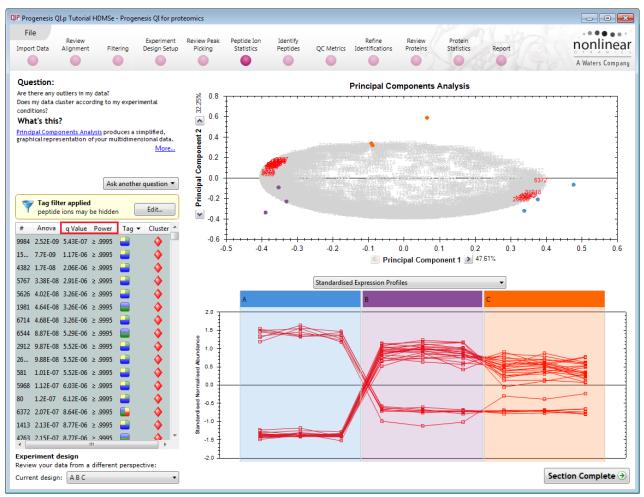
Principal Component Analysis (PCA)

In **Peptide Stats** the first statistically based question asked of the data takes the form of a Quality Control assessment:

Are there any outliers in my data? And does my data cluster according to my experimental conditions?

It answers this question by:

'Using Principal Components Analysis (PCA) to produce a simplified graphical representation of your multidimensional data'.



PCA can be used to determine whether there are any outliers in the data and also look at how well the samples group. The groupings that can be observed on the 2D PCA plot can be compared to your experimental conditions and conclusions can be drawn regarding possible outliers in your data. Selecting peptide ions in the table will highlight the peptide ions on the 'Biplot' and their expression profiles will appear in the lower panel.

Note: the Table in the Stats view contains additional columns:

q value: tells us the expected proportion of false positives if that peptide ion's p-value is chosen as the significance threshold.

Power: can be defined as the probability of finding a real difference if it exists. 80% or 0.8 is considered an acceptable value for power. The Power Analysis is performed independently for each peptide ion, using the expression variance, sample size and difference between the means.

Also, for a given power of 80% we can determine how many samples are required to ensure we find a difference if it actually exists.

Note: Power analysis is discussed in Appendix 6 (page 91)

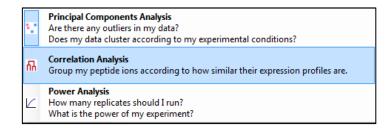
Correlation Analysis

With the tag filter still set to display only the top 4361 **Most Abundant** peptide ions, we are going to explore the Correlation Analysis of these peptide ions.

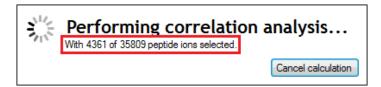
To set up the **Correlation Analysis** using this filtered data set click on **Ask another question** (above the table)

Create a filter Show or hide peptide ions based on a selection of o create the filter. For more guidance, please se	
Available tags:	Show peptide ions that have all of these tags:
Anova p-value ≤ 0.05 (20641 peptide ions)	Most Abundant (4361 peptide ions)
Max fold change ≥ 2 (7890 peptide ions)	Show peptide ions that have at least one of these tags:
Separated by drift time (688 peptide ions)	Hide peptide ions that have any of these tags:

A selection of 3 tools will appear in the form of questions.



Select the second option to explore 'peptide ion correlation based on similarity of expression profiles'



This time the statistically based question(s) being asked is:

'Group my (selected) peptide ions according to how similar their expression profiles are'

The question is answered by:

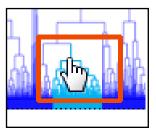
'Using Correlation analysis to evaluate the relationships between the (selected) peptide ions' expression profiles'.

The answer is displayed graphically in the form of an interactive dendrogram where the vertical distance, between each peptide ion can be taken as indicative of how similar the expression profiles of each cluster of peptide ions are to each other.



Correlation Analysis enables the grouping of peptide ions together according to how similar their expression profiles are.

For example: To highlight groups of peptide ions demonstrating **different expression profiles** click on a 'node' for a branch of the Dendrogram (as shown above) while holding the mouse button, hold down the **Ctrl** key and then click on another node as shown.



If you have selected 2 nodes then there will be two expression profile graphs

Note: by highlighting a group of peptide ions with similar expression the peptide ions are identified with the same colour of cluster flag in the table. This allows the table to be sorted on cluster and tagged accordingly

Tip: when reviewing the tags (see above) if you are not applying a new filter then use the **Cancel** button to return to the main view, this prevents unnecessary recalculation of your data.

Before moving to the Identify Peptides stage in the Workflow, first return to the PCA display and clear all tag filters **Clear all Tag filters**.

To move to the next stage in the workflow, Identify Peptides, click Section Complete.

Stage 9: Identify peptides

Progenesis QI for proteomics is designed to perform peptide identifications either directly or by allowing you to export MS/MS peak lists in formats which can be used to perform peptide searches by various search engines. The resulting identifications can then be imported back into Progenesis QI for proteomics, using a number of different file types, and matched to your detected peptide ions.

				QC Metrics Ide	Refine ntifications	Review Proteins	Protein Statistics	Report	R	A Waters Compa
Feat	ures							Vo filte	er applied Cre	@ Hel
#	Identifications	m/z	Charge	Retention time	Drift time	Tag 💌				
• 1	0	828.4192	2	58.67	4.90					
03	0	822.4427	2	69.31	4.76					
04	0	962.7957	3	76.54	4.97	-				
05	0	894.4745	3	67.50	4.48					
0 6	0	964.1703	3	63.92	5.45					
07	0	823.4391	2	75.96	5.04					
08	0	763.7624	3	69.69	4.07					
0 9	0	913.5255	2	71.77	6.28					
									60.4 59.3 58.2 57.1 • Retention Time (min)	m/z
Fragr	nent matches	for:								
Intensity (counts)	v .									
	0 0.05 0	.1 0.15	0.2 0	.25 0.3 0.35	5 0.4 0	.45 0.5 m/z	0.55 0.6	0.65 0.7	0.75 0.8 0.8	5 0.9 0.95
	Fragr	Picking Statistic # Identifications • 1 0 • 3 0 • 4 0 • 5 0 • 6 0 • 7 0 • 8 0 • 9 0 Identifications for j Peak mass Peptide r	Picking Statistics Pep # Identifications m/z • 1 0 828.4192 • 3 0 822.4427 • 4 0 962.7957 • 5 0 894.4745 • 6 0 964.1703 • 7 0 823.4391 • 8 0 763.7624 • 9 0 913.5255 Jdentifications for peptide io Peptide mass Peak mass Peptide mass Fragment matches for: 1 1 1 1 1	Picking Statistics Peptidés # Identifications m/z Charge •1 0 828.4192 2 •3 0 822.4427 2 •4 0 962.7957 3 •5 0 894.4745 3 •6 0 964.1703 3 •7 0 823.4391 2 •8 0 763.7624 3 •9 0 913.5255 2 Identifications for peptide ion 1 Peak mass Peptide mass Protein mass	Picking Statistics Peptides QC Metrics Ide * Identifications m/z Charge Retention time • 1 0 \$28,4192 2 \$56,67 • 3 0 \$822,4427 2 \$99,31 • 4 0 962,797 3 76,54 • 5 0 \$98,44745 3 63,92 • 7 0 \$23,4391 2 75,96 • 8 0 763,7624 3 69,69 • 9 0 913,5255 2 71,77 Identifications for peptide ion 1 Peak mass Peptide mass Mass Error (Da)	Picking Statistics Peptides QC Metrics Identifications # Identifications m/z Charge Retention time Drift time • 1 0 828.4192 2 58.67 4.90 • 3 0 822.4427 2 69.31 4.76 • 4 0 962.9797 3 67.50 4.49 • 5 0 894.4745 3 63.92 5.45 • 7 0 823.4391 2 75.96 5.04 • 9 0 913.5255 2 71.77 6.28 Identifications for peptide ion 1 Peak mass Peptide mass Protein mass Mass Error Identifications for: Identifications for:	Picking Statistics Peptide QC Metrics Identifications Proteins # Identifications m/z Charge Retention time Drift time Tag Image: Charge Drift time Tag Image: Charge Statistics Statistics	Picking Statistics Peptidés QC Metrics Identifications Proteins Statistics Features Telenis Difference Statistics Peptidés QC Metrics Identifications Proteins Statistics * Identifications m/z Charge Retention time Drift time Tag * • 1 0 828.4192 2 58.67 4.90 •<	Picking Statistics Peptides QC Metrics Identifications Proteins Statistics Report Features Image: No filt No filt No filt No filt No filt * Identifications m/z Charge Retention time Drift time Tag Image: No filt * Identifications m/z Charge Retention time Drift time Tag Image: No filt * Identifications m/z Charge Retention time Drift time Tag Image: No filt * Identifications m/z Charge Retention time Drift time Tag Image: No filt * Identifications m/z Statistics Report No filt * Identifications Mass Statistics Report * Identifications Seq Seq Seq Seq * Peak mass Peptide mass Protein mass Mass Error (Da) Mass Error (ppm) Score Seq. start	Picking Statistics Peptide QC Metrics Identifications Proteins Statistics Report Features * Identifications m/z Charge Retention time Drift time Tag *

For this example we are using the direct method **Ion Accounting**.

Note: Following the full automatic processing, described in Stage 2 of this guide, the Identify Peptides page currently displays the full list of the detected peptide ions in your experiment and some of their attributes, including the number of **Identifications** (as shown on page 51). If search results exist these can be cleared by clicking **Clear all identifications**, this will allow you to re-perform the search.

Entering Search Parameters	Identify Peptides
	Select your peptide identification method:
irstly you need to select the FASTA file containing peptide and rotein identifications.	Q Ion Accounting
	For MS ^s , HDMS ^s and SONAR data
WISSPROT-1 is provided with the installation of the software.	1 Enter the search parameters Select your FASTA file containing peptide and protein identifications:
o add new Databanks in the form of FASTA files click on Edit o open the Databank editor	SWISSPROT-1.0 Edit SWISSPROT-1.0 STANDARD_SPACED Common search parameters
lote: the SWISSPROT-1.0 is locked	Search tolerance parameters
	 Ion matching requirements

QIP Databank Editor		X
	Name:	SWISSPROT-1.0
B SWISSPROT-1.0	Parsing rules:	STANDARD_SPACED -
	Location:	sprot.fas
Add Remove		
		Save Cancel

For a new Databank you need to give it name, select the parsing rules and specify the location of the FASTA file, see the example below.

QIP (Databank Editor		×
۵	Name	Name:	Tutorial DB
â		Parsing rules:	
	Tutorial DB	Location:	> v4 HDMSe Tutorial\Tutorial_nd_DB.fasta
		Location:	o v4 HDMSe Tutorial\Tutorial_nd_DB.fasta
	Add Remove		
			Save Cancel

The new Data bank will appear in the left panel now click **Save** to return to the Search parameters.

If your databank is not already displayed then select it from the drop down list.

Expand the Common search parameters

The default settings are displayed:

Digest reagent: is set as Trypsin. Alternative Digest reagents are available from the list and additional ones can be added to the list using the **Reagent editor...**

Missed cleavages: is set as 1.

Maximum protein mass: is set at 250kDa

Modifications: are set Carbamidomethyl C (Fixed) and Oxidation M (variable). More modifications are available from the list and additional ones can be added to the list using the **Modification** editor...

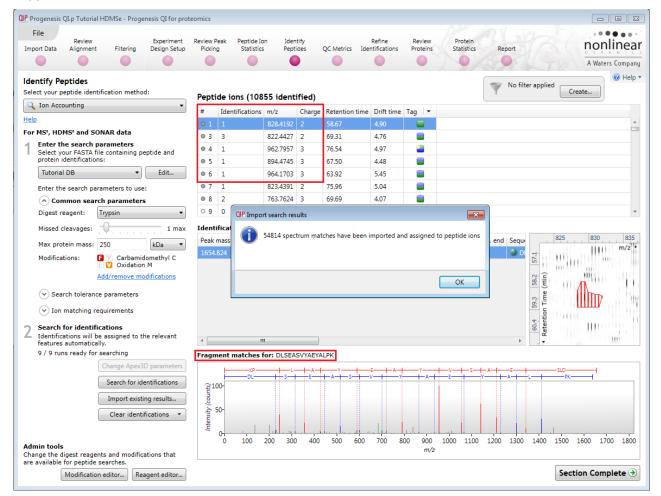
Identify Peptides	
Select your peptide ide	ntification method:
Q Ion Accounting	•
Help	
For MS ¹ , HDMS ¹ and	SONAR data
1 Enter the search Select your FASTA protein identificati	file containing peptide and
Tutorial DB	▼ Edit
Enter the search p	parameters to use:
🕑 Common sea	rch parameters
🛛 Search tolera	nce parameters
 ✓ Ion matching 	requirements
	- b b
\bigcirc	rch parameters
Digest reagent:	Trypsin 🔹
Missed cleavages:	1 max
Max protein mass:	250 kDa 🔻
Modifications:	Image: Carbamidomethyl C
	Add/remove modifications

Having selected the Databank then set the Search tolerance parameters and ion matching parameters as shown to the right.

When all the runs are ready for searching, click **Search for identifications**.

Depending on the search parameters and the number of runs the **Ion Accounting** search can take some time.

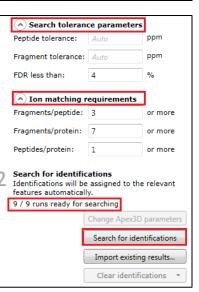
Once the Ion Accounting is complete, peptide ions with identifications are identified with a solid grey symbol and the number of identifications appears in the next column.



Details for the current peptide ion identifications are displayed in the table below and the Fragment ions for the current identification are displayed in the bottom panel.

Note: if you want to perform the search with a new set of parameters then first select Clear all identifications

Having performed the process of peptide identification you can review the overall quality of your analysis by using the various batch-based quality metrics in the next section, by clicking on QC Metrics on the workflow.



Stage 10: QC Metrics

Progenesis QI for proteomics includes a number of batch-based QC metrics which you can examine to increase confidence in, or identify issues with, your data. These views are presented at the "QC Metrics" page. By setting up experimental groupings that correspond to your batch metadata, you are able to investigate whether there are any systematic effects associated with your processing. This gives you confidence that your conclusions are not affected by technical biases within the course of the experiment.

The charts are updated with changes in your active experimental design, allowing you to examine the effects of all stages of your processing if you wish through using different batch divisions.



Metrics

There are two sets of metrics. Firstly, the overview page provides nine at-a-glance summary measurements which cover the experiment as a whole. Then, the remainder of the pages provide more detailed information and bring in the batch-by-batch detail, some of which builds on the overview charts:

- Sample preparation metrics highlighting issues or problems with the preparation of your samples: Missed cleavages, modifications and abundance dynamic range
- Instrument metrics highlighting whether your chromatography column and mass spectrometer are configured and performing correctly: Mass accuracy, abundance dynamic range (again), precursor charges, MS1 scan rates
- Experiment metrics concerning the identified proteins and peptides in your experiment, allowing you to pick out any outlying runs or conditions:

Proteins, peptides, peptides per protein, % of peptide ions identified, proteins per condition

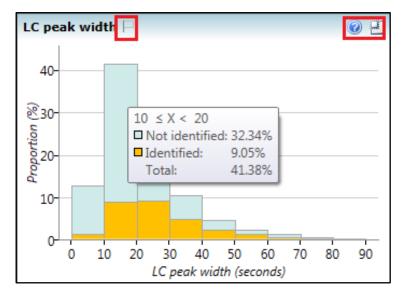
More detailed information on the QC metrics is available on the FAQ pages.

Interpretation and use

It's worth noting that there is no simple 'right' or 'wrong' answer as to whether your data are of high enough quality.

Note: QC measurements are designed to flag up potential issues for investigation, hence there must be an assignable cause to the variation observed to render QC practical.

Furthermore, the QC metrics will be rebuilt if you delete and re-do any stages leading up to them (for example, clearing identifications and re-searching with changed identification parameters, or altering the alignment). Hence, if you archive your experiment before and after your changes, or export the charts before and after, you can see the effects of your changes upon the quality measurements – a very useful method for assessing your interventions.



Note: that the overview metrics can each be expanded by clicking on the top-right icon in the sub-window. Additionally, hovering over a column will bring up a tooltip containing quantitative information on the results.

You can also tag metrics of interest or concern with a comment for your records, which is saved with the experiment. To do this, click the empty flag icon in the tab header for the given metric (or in the tile header on the overview screen):

Missed cleavages	\square	PTMs 📄	Abundance dynamic range 🖂		Identifications overview	F	. 0 🗄
------------------	-----------	--------	---------------------------	--	--------------------------	---	-------

This will pop up a dialog allowing you to enter a comment describing why this metric has been flagged.

Reporting

You can export a report for your own records, or to pass on to another member of your team:

3	Produce a report Create a report for either the flagged metrics, or for all metrics.
	Report flagged metrics Report all metrics

Report all metrics:

Generates a report containing all metrics shown in the application (overview, sample preparation metrics, instrument metrics and experiment metrics). This may be useful for documentation purposes, or to verify the quality of your experiment.

Report flagged metrics:

Generates a report of only metrics you have flagged, along with the message you provided. This may be useful for giving to a technician or other team member, to highlight areas of the experiment that need improvement.

In order to review, and refine the quality of the **Peptide Search** results click on the next stage in the workflow, **Refine Identifications**.

Stage 11: Refine Identifications

In this example we are going to apply a number of filters to 'refine' the quality of the Databank search.

Note: before removing any identifications, make sure there are **no** tag filters applied at the Identify peptides stage.

As an **example** the following section describes how sequential filtering of the Peptide results can be performed using the following thresholds described below:

- Remove identifications with a Score less than 4
- Remove identifications where less than 2 hits were returned
- Remove all identifications where the Protein Description **Contains** the following: 'Putative', 'Probable', 'Like', 'Potential' and 'Predicted'

To perform these filters, on the Batch detection options panel, set the Score to less than 4, then **Delete matching search results**.

File	Review Alignment	Filtering	Experiment Design Setup	Review Pea Picking	k Peptide I Statistic		dentify eptides	QC Metric		Refine tifications	Revie Protei		Report			nonline
•				•	•		•				•		0			A Waters Comp
efine Ide	entifications			Batch o	leletion criteri	a					_					
	ide identificatio sults, you can i					Score	less than	•	4			Seq	uence Length	less than 🔻		
Specify	y a set of dele	tion criteria				Hits	less than	•					Charge	less than 🔻		
	batch deletion of for a set of ider					Mass	less than	•					Sequence	contains 🔹		
delete.		initiations y		Absolu	te mass erro	(mag)	less than	•					Accession	contains 🔻		
Delete	the unwante	d identificat	tions				less than	-					Description			
To delet either:	te the identifica	ations you do	n't want, click													
• De	lete Matching S		s, to delete		Retentio	n Time	less than	•					Modifications	contains 🔻	· [
• Del	e highlighted ID lete Non-match	ning Search F										Delete matching :	search results	Delete non-mate	ching search result	Reset the criteri
del	lete the IDs tha	it are <i>not</i> hig	hlighted		# Score	Hits	m/z	RT(mins)	Charge	Mass	Mass ern	Sequence	Accessio	n Modificat	tions	
	the criteria to ify another bat			14	8 3.67	1	665.86		2	1329.7(ILKEMPSE	C4YI91		Putativ	e uncharacterized p
delete,	click Reset the			V 15	4.34	1	658.36	49.10	2	1314.71	-27.97	NLVSGSTV	C4YJK7			/e uncharacterized p
step 1 a	above.			15	4 4.56	5	658.36	49.10	2	1314.71	-11.92	ILGPGNQF	O74712		Histidii	ne biosynthesis trifur
🤝 No f	filter applied]	15	4 8.32	2	658.36	49.10	2	1314.71	8.24	EQLESQLV	C4YQP9		60S rib	osomal protein L35
Υ		l	Create	15	5		830.46	56.33	2	1658.91	15.68	VINEPTAA	C4YK39		Heat sl	hock protein SSC1_ r
T-4-	al Hits m/z	DT (min Ch		V 15	5 5.02	1	830.46	56.33	2	1658.91	8.93	🔇 SNPIFTKN	C4YS46		Putativ	e uncharacterized p
Tota		RT (min Ch	arge Tag	V 15	5 8.48	8	830.46	56.33	2	1658.91	15.68	🔇 IINEPTAAA	P46587		Heat sh	hock protein SSA2 G
. 7	828.419	58.67 2		V 15	5 8.47	5	830.46	56.33	2	1658.91	15.68	🎯 IINEPTAAA	P10591		Heat sl	hock protein SSA1 O
11	822.442	69.31 2		V 15	5 5.02	1	830.46	56.33	2	1658.91	8.93	SNPIFTKN	Q5AJY6		Likely 2	26S proteasome regi
9	962.795	76.54 3		V 15	5 8.48	8	830.46	56.33	2	1658.91	15.68	IINEPTAAA	P41797		Heat sl	hock protein SSA1 G
8	894.474	67.50 3	•	V 15	8 3.69	1	543.98	44.89	3	1628.91	21.88	QALSETVD	C4YKI9		Putativ	e uncharacterized p
8	964.17(63.92 3		V 15	8 3.73	1	543.98	44.89	3	1628.91	8.86	STLTEINGP	Q5A9Y0		Putativ	/e uncharacterized pr
7	823.439	75.96 2	•	16	0.00	1	618.84	50.83	2	1235.67	42.52	GLSYSKDE	C4YIX5		Putativ	/e uncharacterized pi
11	763.762	69.69 3		V 16	5.71	1	618.84	50.83	2	1235.67	-8.39	S FDNGLRFIVR	Q5A202		Putativ	e uncharacterized pr
0 10	657.367	36.18 2		V 16	1 8.59	4	618.84	50.83	2	1235.67	13.06	STLASDGIA	Q5A516		ADP_A	TP carrier protein GN
1 19	920.144	74.48 3		16	5 8.11	9	697.37	56.25	2	1392.72	7.96	ALDADVVS	P82610		5-metł	hyltetrahydropteroyli
39	828.449	45.13 2		✓ 16	6 5.32	1	965.48	51.10	2	1928.95	8.09	AVSSGMV	C4YEB6		Glycyl-	tRNA synthetase 1 G
6 13	776.411			V 16	6 8.10	8	965.48	51.10	2	1928.95	11.92	S VWLDPNE	C4YQK4		60S rib	osomal protein L19
9 6	740.478	52.50 2		V 16	7 4.56	3	821.96	50.91	2	1641.9(11.73	LASESTLPV	C4YSQ4			ve uncharacterized pr
			•	-								~	•			÷
					arch results. 1										_	

Note: the search results matching the filter criteria turn pink and the total is displayed at the bottom of the table (in this example: 1992 matching out of 14747)

 IP Delete 1992 search results?

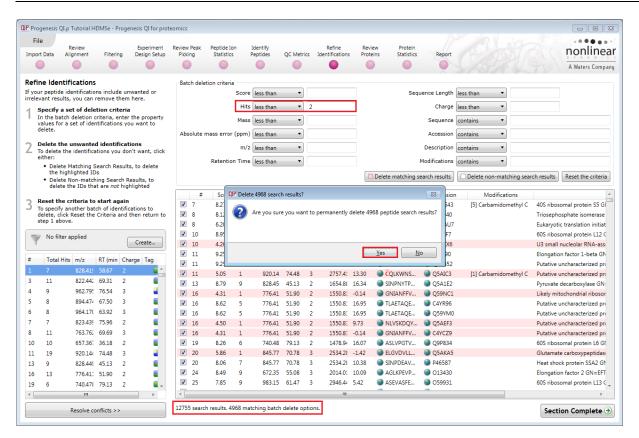
 Image: A re you sure you want to permanently delete 1992 peptide search results?

 Image: Yes

Note: a dialog warns you of what you are about to delete Click **Yes**.

Now click Reset the criteria to clear the previous threshold then apply the next filter 'Hits: less than 2'.

Tip: always click Reset the Criteria after each deletion.



Now in the Description first enter 'Like' and delete matching search results. Then enter the 'regular expression': regex: Puta|Prob|Pote|Pred and delete matching search results.

QIP Proger	nesis QI.	p Tutorial Hl	DMSe - Pro	genesis QI for prot	teomics										
File															
Import D	ata	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ior Statistics	Identify Peptides	QC Metrics	Refine Identifications	Review Proteins	Protein Statistics	Report			nonlinear
Import D		Alignment	rittering	Design Setup	Picking	Statistics	Peptides	QC Metrics		Proteins	Statistics	Report			D Y N A M T C
									•				A PACK	T	A Waters Company
Refine	ldent	ifications			Batch del	etion criteria									
		identification		unwanted or			Score less than	•			Seque	ence Length	less than 🔹		
		set of dele					Hits less than	•				Charge	less than 🔹		
Int	he bato	ch deletion c	riteria, ent	er the property			Mass less than	•				Sequence	contains 🔻		
valu dele		a set of iden	tifications	you want to											
					Absolute	mass error (ppm) less than	•				Accession	contains 🔹		
		e unwanteo he identifica		ations don't want, click			m/z less than	•				Description	contains 🔹	regex: Puta	Prob Pote Pred
eith						Retention	Time less than	•			м	Iodifications	contains 🔹		
•		9 Matching S ghlighted ID		ilts, to delete											esults Reset the criteria
•		Non-match the IDs tha				_					lete matching sea	arch results	Delete non-match	ning search ri	suits Reset the criteria
	derete	103 110	e ure not n	igningriced	#	s QIP	Delete 2158 sear	h results?				88 ession	n Modificatio	ons	*
		criteria to another bate			▼ 1	9.1						LV6		40	IS ribosomal protein S26-
dele	ete, clic	k Reset the		d then return to	✓ 3	8.4	🖉 Are you su	re you want t	o permanently d	elete 2158 pe	eptide search res	ults? W54		40	S ribosomal protein S22 (
step	p 1 abo	ve.			V 3	5.2						CM1		Pu	tative uncharacterized pro
	No filte	r applied			☑ 4	9.:						.DM7		G	yceraldehyde 3-phosphat
T				Create	✓ 5	9.0				V	No	CU4	[14] Carbamidor		IS ribosomal protein L2 GI
#	Total Hi	its m/z	RT (min	Charge Tag	✓ 6	8.0				Yes	INO	DM7			yceraldehyde 3-phosphat
1	7	828.419			7	8.1.						G43	[5] Carbamidom	1 A A A A A A A A A A A A A A A A A A A	IS ribosomal protein S5 GI
3	11	822.442	69.31 2	2	 ✓ 8 ✓ 8 	8.12 6.20	9 763.7 2 763.7		3 2288.27 3 2288.27	-	ANVDGFLV	Q9P940 Q9P940 Q5AAU7			iosephosphate isomerase
4 9	9	962.795	76.54 3	3	10	8.95	9 657.3		2 1312.7;	_	AVGGEVG	Q5AJF7			karyotic translation initiat IS ribosomal protein L12 (
5 8	8	894.474	67.50 3	3 🗧	10	9.25	9 920.1		3 2757.41			P78590			ongation factor 1-beta GN
6	8	964.17(63.92	3	11	9.25	9 920.1		3 2757.41	-		Q5A652			tative uncharacterized pre
7	7	823.439	75.96 2	2	13	8.79	9 828.4	5 45.13	2 1654.88	16.34 🔇		Q5A1E2			ruvate decarboxylase GN
8	11	763.762	69.69	3 🚺	V 16	8.62	5 776.4	1 51.90	2 1550.81	16.95 🔇	TLAETAQE	C4YR96		Pu	tative uncharacterized pre
10 :	10	657.367	36.18 2	2	V 16	8.62	5 776.4	1 51.90	2 1550.81	16.95 🔇	TLAETAQE	Q59YM0		Pu	tative uncharacterized pre
11 :	19	920.144	74.48	3 🧧	1 9	8.26	6 740.4	8 79.13	2 1478.94	16.07 🔇	ASLVPGTV	Q9P834		60	IS ribosomal protein L6 GI
13 9	9	828.449	45.13 2	2	20	8.06	7 845.7	7 70.78	3 2534.28	_		P46587		He	eat shock protein SSA2 GN
16	13	776.411	51.90 2	2	24	8.49	9 672.3		3 2014.01			O13430			ongation factor 2 GN=EFT
19 (6	740.478	79.13 2	2 🚺 🗸	25	7.85	9 983.1	5 61.47	3 2946.44	5.42	ASEVASFE	O59931		60	IS ribosomal protein L13 C 🛫
•					-										÷.
		Resolve co	onflicts >>		7454 searc	h results. 215	8 matching batcl	n delete optic	ins.					[Section Complete 🤿

Having applied all the filters there will be 5296 search results remaining

To validate the Peptide search results at the protein level select Resolve Conflicts (bottom left).

Resolving Conflicts

This stage allows you to examine the behaviour of the identified peptides and choose to resolve any conflicts for the various peptide assignments at the protein level.

The **Resolve Conflicts** stage is now accessed the bottom left of the Refine Identifications stage.

The number of conflicts you have to resolve will depend on the scope and stringency of the filters you apply at the **Refine Identifications** stage.

Details on performing conflict resolution are available in Appendix 7 (page 92)

For this guide: we will NOT resolve the conflicts.

The following pages describe the handling of Protein grouping and Protein Quantitation. Options for these are available at both Resolve Conflicts and Review Proteins

Note: the default settings are to Use Protein Grouping and Relative Quantitation using Hi-N

Protein Grouping

QP Protein quantitation options
Quantitation method:
Relative Quantitation using Hi-N
Number of peptides to measure per protein (N): 3
Use protein grouping .e. hide proteins whose peptides are a subset of another protein s.
OK Cancel

Where proteins are identified containing the same peptides then they are effectively indistinguishable aside from score. Also where one protein contains only peptides that represent a subset of another protein's peptides, the protein with fewer peptides can be subsumed into that with the greater number.

With protein grouping switched on (default setting) protein groups and the additional members are indicated by a bracketed number located after the Accession number. Taking **Glutathione reductase (C4YR40)** as an example, we can look at the other group members by changing the protein options.

Accession	Peptides	Unique	Conflict:	Score	Tag	• *		#	Σ	ę	Score	Hits	Correlation	Mass	Mass error (p.	RT (mins)	Charge	Tag	▼ Abundance	Confl	ict: ^
C4YNX7	12	12	0	120		_	1	/ 10	006	• 3	6.41	7	0.292	1100.643	-1.19	42.3	2		6.57E+04	0	Ξ
C4YR40 (+2)	o 12	12	0	77.4				/ 12	86	2	5.73	2	0.338	1092.59	8.18	42.2	2		5.74E+04	0	
S 042766 C4Y	R40 - Glutathic	one reduc	tase GN=C	AWG_04539	PE=4 SV=	1	3	61	L 70 ©	0	6.72	9	0.986	1195.694	0.952	50.1	2		1.76E+04	0	
S C4YDN4	12	11	1	83.5		-		66	5 51 C	1	6.51	7	0.835	1393.662	9.94	41.5	2		1.15E+04	0	-
•						P.							111								P.
Peptide ion views Conflicting		L					Pept	ide i	ions o	f co	onflictin	g protei	in								
Conflicting r	oroteins						Pent	ide i	ions o	fro	onflictin	a protei	in								
Accession	Peptides Ur	nique (Conflict: Pr	otein Score	Pe	epti	-	ŧ	ΣĢ	ъ	Score	Hits C	orrelation N	Aass N	lass error (p	RT (mins)	Charge T	ag 👻	Abundance	Conflict:	Pept
٠						F	•						III								Þ
€ Refine Ide	ntification	Pro	tein option:	s															Section Com	plete	۲

Although Conflict resolution has not been performed this protein has no conflicting peptides.

Protein grouping is switched on b click on Protein options (botto option.	QIP Protein quantitation options Quantitation method: Relative Quantitation using Hi-N Number of peptides to measure per protein (N): 3				
As grouping is switched off the gr conflicts to the other group memb		Use protein grouping of another protein's.	hose peptides are a subset		
Accession Peptides Unique Conflict: Score Tag ▼ Al ▲ C4YE07 12 11 3 62 3.2			Abundance Conflict: ▲ 6.57E+04 2		
		1100.643 -1.19 42.3 2			
Q Q5A2T2 12 12 0 95.3 8.0	☑ 24704 ○ 0 6.9 7 0.387 ☑ 10105 ○ 0 500 □ 0 0.011	1774.878 2.16 70.1 2 972.551 1.7 54 2	4.92E+03 2		
Q Q5A017 12 11 2 132 11	E 10105 0 0105 / 01511	51 2	1.11E+04 2		
	✓ 6651 ○ 1 6.51 7 0.835	1393.662 B ^{9.94} 41.5 2	1.15E+04 2 👻		
			4		
Protein: C4YR40 Glutathione reductase GN=CAWG_0453					
Protein: Q5W322 Glutathione reductase GN=glr1 PE=3 SN	/=1				
Peptide ion views Protein resolution					
Conflicting proteins for peptide ion 1006	Peptide ions of Q5W322	F			
Accession Peptides Unique Conflict: Protein Score Pepti	# Σ ⊡ Score Hits Correlation I	Mass Mass err or (p RT (mins) Charge Tag	✓ Abundance Conflict: F ▲		
S C4YR40 ○ 12 0 21 77.4	☑ 1006 ○ 3 6.41 7 0.292 1	.100.643 -1.19 42.3 2 🥃	6.57E+04 2 🔮 _≡		
S Q5W322 12 0 21 77.4		.774.878 2.16 70.1 2 🥃	4.92E+03 2 🔇		
Q9UV69 9 0 18 58.1 6.41	☑ 10405 ○ 0 6.83 7 0.911	972.551 1.7 54 2 📒	1.11E+04 2 🔮		
	✓ 6651 ○ 1 6.51 7 0.835 1	.393.662 9.94 41.5 2 🥃	1.15E+04 2 🔇		
	☑ 8786 ○ 0 6.66 9 0.654 1	.033.589 8.25 57 2 🥃	1.14E+04 2 🔇 🖕		
۰	•		Þ		
Refine Identifications Protein options			Section Complete		

Note: when the grouping is switched off the other group members appear in panel E showing conflicts with the other group members and **no** Unique peptides

Before moving to **Review Proteins** it is worth understanding the available options to handling Protein Quantitation in Progenesis QI for proteomics.

QP Protein quantitation options						
Quantitation method:						
Relative Quantitation using Hi-N						
Number of peptides to measure per protein (N):						
Use protein grouping .e. hide proteins whose peptides are a subset of another protein's.						
OK Cancel						

The next section in this guide describes the use of alternatives to the default method shown to the right.

Note: If you are using the default method, you can go directly to the **Review Protein** stage page 61 using the workflow.

Protein Quantitation options

There are 5 options with which to control how the Protein Quantification is performed by Progenesis QI for proteomics.

The default option that will be applied is **Relative Quantitation using Hi-N**.

If you have selected one of the other options during the setup of the Auto Processing of your data (Stage 1) then this option will be applied.

Hi-N in Progenesis QI for proteomics is an implementation of Hi-3 as described by Silva *et al.* [References]. After peptide and protein identification, the abundance of each peptide is calculated from all its constituent peptide ions.

For each protein, the N most abundant peptides (N being set according to the user selection) have their **abundances averaged** to provide a reading for the protein signal.

The ranking of peptide abundance is based on the integrated value across all the runs, allowed by the accurate alignment and lack of missing values. This gives added confidence in the peptide selection, taking all runs into account to make the ranking robust.

IP Protein quantitation options						
Quantitation method:						
Relative Quantitation using Hi-N						
Absolute Quantitation using Hi-N Requires a calibrant protein to calculate absolute amounts Uses mean calibrant abundance measured across runs Uses the most abundant N peptides Allows comparison between proteins within a run 						
Relative Quantitation using Hi-N Uses the most abundant N peptides Allows comparison between proteins within a run						
Relative Quantitation using non-conflicting peptides • Uses only peptides which have no conflicting protein identifications • Allows comparison of a single protein across runs						
Relative Quantitation using all peptides • Uses all peptides identified as part of a protein • Allows comparison of a single protein across runs						
Absolute Quantitation for HCP using Hi-N Requires a calibrant protein to calculate absolute amounts Uses calibrant abundance measured per run Uses the most abundant N peptides Calculates the amount of HCPs per run in fmol and ng 						

The averaged abundance readings not only make possible the **relative quantitation** of the same protein across all runs to be determined but also allow, with the inclusion of a known amount of a calibrant protein in each run, this to be converted to an **absolute** reading for protein amount.

To generate values for Absolute Quantitation enter the accession number and amount for the calibrant.

QP Protein quantitation options		QIP Protein quantitation options
Quantitation method:		Quantitation method:
Absolute Quantitation using Hi-N		Absolute Quantitation using Hi-N
Number of peptides to measure per protein (N):		Number of peptides to measure per protein (N):
Calibrant accession:		Calibrant accession: P83773
Amount (fmol):		Amount (fmol): 50
Use protein grouping i.e. hide proteins whose peptides are a subset of another protein's.	, , , , , , , , , , , , , , , , , , ,	Use protein grouping i.e. hide proteins whose peptides are a subset of another protein's.
OK Cancel		OK Cancel

The absolute amounts, based on the calibrant used, are reported at the Review Proteins stage as additional columns (one for each condition) following the protein description.

Review Proteins Using this screen, you can find the proteins of interest in your experiment.	Vo filter applied Create			1 He
Set the quantitation options	Description	Amount (fmol) - A	Amount (fmol) - B	Amount (fmol)
If you've not already done so, choose between relative and absolute guantitation,	Glyceraldehyde 3-phosphate dehydrogenase GN=TDH3 PE=3 SV=1	477	800	852
use of Hi-N, protein grouping and more.	Glycerol-3-phosphate dehydrogenase GN=GPD2 PE=3 SV=1	79	140	121
Protein options	Glycerol-3-phosphate dehydrogenase_ mitochondrial GN=GUT2 PE=3 SV=1	30.3	33.5	33.1
	Glycine cleavage system H protein GN=CAWG_00084 PE=4 SV=1	27.1	37.4	31.8

The ranking of peptide abundance is based on the integrated value across all the runs, allowed by the accurate alignment and lack of missing values. This gives added confidence in the peptide selection, taking all runs into account to make the ranking robust. These Hi-N methods allow the relative and absolute comparison of proteins within the same run.

Note: When there are **peptide conflicts** (a peptide is shared between two proteins, for example) it is important to assign the signal correctly for absolute quantitation. To do this, Progenesis QI for proteomics carries out a two-step process. Firstly, Hi-N is carried out only on the N most abundant **unique** (non-conflicting) peptides of the proteins concerned. This provides a ratio estimate for the two proteins based only on unshared peptides. The abundance of any shared peptides is then divided and allotted in this ratio between the two proteins, and the full Hi-N calculation is then applied using the divided values for conflicted peptides.

Naturally, if the conflicted peptides are not among the N most abundant in either protein initially, then this will not cause any difference in the result.

To generate values for **Absolute Quantitation for HCP** (Host Cell Proteins) select the 5th option on the drop down at the beginning of this section.

Note: there are 2 differences between this HCP-specific option and the Absolute Quantitation option:

- The mass of protein present is also reported in ng in addition to the amount in fmol
- The calculation of protein amounts and masses present is based on measurements of the calibrant protein present in the same run, and not a pooled measurement over all the runs. It does still assume a specified amount of a calibrant in every run. However, it does not pool information across the runs to derive its relationship between the calibrant and observed abundance. The amount of a contaminant is instead calculated directly by relation to the observed amount of the calibrant in the same run.

Review Proteins	S No filter applied						
Using this screen, you can find the proteins of interest in your experiment.	Create						
Set the quantitation options	Description	Amount (fmol) - A	Amount (fmol) - B	Amount (fmol) - C	Amount (ng) - A	Amount (ng) - B	Amount (ng) -
If you've not already done so, choose between relative and absolute guantitation,	Glyceraldehyde 3-phosphate dehydrogenase GN=TDH3 PE=3 SV=1	471	724	971	16.9	26	34.9
use of Hi-N, protein grouping and more.	Glycerol-3-phosphate dehydrogenase GN=GPD2 PE=3 SV=1	78.1	127	138	3.22	5.22	5.68
Protein options	Glycerol-3-phosphate dehydrogenase_ mitochondrial GN=GUT2 PE=3 SV=1	29.9	30.3	37.7	2.18	2.2	2.75
	Glycine cleavage system H protein GN=CAWG_00084 PE=4 SV=1	26.8	33.8	36.1	0.548	0.692	0.739

As for Absolute Quantitation using Hi-N, enter the accession number and amount for the calibrant.

QP Protein quantitation options		QP Protein quantitation options
Quantitation method:		Quantitation method:
Absolute Quantitation for HCP using Hi-N		Absolute Quantitation for HCP using Hi-N
Number of peptides to measure per protein (N):		Number of peptides to measure per protein (N):
Calibrant accession:		Calibrant accession: P83773
Amount (fmol):		Amount (fmol): 50
Use protein grouping i.e. hide proteins whose peptides are a subset of another protein's.	, , , , , , , , , , , , , , , , , , ,	Use protein grouping i.e. hide proteins whose peptides are a subset of another protein's.
OK Cancel		OK Cancel

The absolute amounts, based on the calibrant used, are reported at the Review Proteins stage as additional columns (one for each condition) following the protein description.

How the values for the calibrant behave across runs comparing Absolute Quant with Absolute Quant for HCP using the same calibrant (Acetyl-CoA hydrolase)

Description	Amount (fmol) -	A Amount (fmol) - B	Amount (fmol) - C			
Acetolactate synthase small subunit_mitochondrial GN=CAWG_03647 PE=4 SV=1	23.1	26	30.7			
Acetyl-CoA hydrolase GN=ACH1 PE=1 SV=2	50.6		44			
Acetyl-coenzyme A synthetase 1 GN=ACS1 PE=3 SV=1	6.66	8.89	7.95			
Description	Amount (fmol) -	A Amount (fmol) - B	Amount (fmol) - C	Amount (ng) - A	Amount (ng) - B	Amount (ng) - C
Description Acetolactate synthase small subunit_ mitochondrial GN=CAWG_03647 PE=4 SV=1	Amount (fmol) - 22.9	A Amount (fmol) - B 23.5	Amount (fmol) - C 35	Amount (ng) - A 0.764	Amount (ng) - B 0.785	Amount (ng) - C 1.17

Relative Quantitation can also be performed comparing a single protein across all the runs using only the unique or non-conflicting peptides. Select the third method from the drop down

Using non conflicting peptides

QP Protein quantitation options							
Quantitation method:							
Relative Quantitation	using non-conflicting peptides	•					
Use protein grouping i.e. hide proteins whose peptides are a subset of another protein's.							
	OK	Cancel					

The relative Quantitation can also be performed using all peptides.

OP Protein quantitation options						
Quantitation method:						
Relative Quantitation	using all peptides	•				
Use protein grouping of another protein's.	i.e. hide proteins	whose peptides are a subset				
		OK Cancel				

Note: if you have performed conflict resolution then there will be no difference between these methods.

Make sure that the Protein quantitation method is set to Relative Quantitation using Hi-N (where N=3) and Use protein grouping is ticked.

Now move to the **Review Proteins** section by clicking on the icon on the workflow at the top of the screen.

Stage 12: Review Proteins

The **Review Proteins** stage opens displaying details for all proteins. You can now create tags at the level of the proteins. Right click on the table and create Quick Tags for proteins with an Anova p value ≤ 0.05 and Max Fold change ≥ 2 .

		Identify Peptides QC				Protein Statistics	Report	34	R	nonlinea
-			•							A Waters Compa
W No filter	applied	Create	earch	٩						@ Hel
Accession	Peptides	Unique peptides	Confidence score	Anova (p)	g Value	Tag 💌	Max fold change	Highest Mean	Lowest Mean	Description
O9P457	4	4	31	1.36E-06	8.73E-06		1.95	-	A	Cu-binding metallothi
	3	3	22.9	2.13E-06	1.04E-05		4.1		Δ.	Sorbose reductase SO
O59W63	2	1	11.1	2.65E-06	1.04E-05		1.35	A	c	Ribosomal RNA assem
		3	24.8	2.84E-06	1.04E-05		1.78	B	Δ	D-arabinitol 2-dehydro
•	-									Isocitrate lyase GN=IC
			No tags to assign							Cell surface hydrophol
•		-	New tag							Phosphoenolpyruvate
		-	Quick Tags	•		a p-value				Dihydroxy-acid dehyd
-			Edit tags	_	Max f	old change.				Proteasome compone
			-	v	Modi	fication				Glycine cleavage syste
-		-			Seque	ence		-		1_3-beta-glucan synth
-		-			Peptie	de tags cont	ain	-		Carnitine O-acetyltran
-					2145-05		27			Inositol-3-phosphate
-										Protein translation fac
		-							-	Transketolase 1 GN=T
-	25	25		1.710-05	2.140-03		1.//	D	A	Transketolase 1 Giv=1
		-	yase GN=ICL1	PE=3 S	5V=1					
i 11.5 -		A				В			С	
ArcSinh Normalised Abunda 0.05 100 100 100 100 100 100 100 100 100 1		i¢i				M			*	I
*										
	Review Peak Pepi State Picking No filter at Accession Q 9P457 P 87219 Q 59W63 Q 72828 Q 72828 Q C4YNC2 (+1) Q C4YK9 Q C4YN11 Q C4VHP3 Q 752828 Q 4VHP3 Q 504089 Q 504089 Yearboard Q 59409 Selected pr View peptide mean View peptide mean 11.0	Review Peak Picking No filter applied Accession Peptides QPP457 4 P87219 QP9827 P43066 3 QP9827 P43066 3 QP9827 Q7288 3 C4YNC2 (+1) 9 C4YS49 3 Q254E8 4 C4YNC2 (+1) 9 C4YS49 3 Q254E8 4 C4YNC2 (+1) 9 C4YS49 3 Q254E8 4 C4YNC2 (+1) 9 C4YS49 3 Q254E8 4 C4YNC2 (+1) 9 C4YS49 3 Q254E8 4 C4YNC2 (+1) 9 C4YS49 3 Q254E8 4 C4YNC2 (+1) 9 C4YS49 3 C4YNC2 (+1) 9 C4YS49 3 C4YNC2 (+1) 9 C4YS49 3 C4YNC2 (+1) 9 C4YS49 3 C4YNC2 (+1) 9 C4YS49 3 C4YNC2 (+1) 9 C4YS49 3 C4YNC2 (+1) 9 C4YS49 3 C4YNC2 (+1) 9 C4YS49 2 C4YNC3 C	Review Peak Peptide Ion Identify Peptides QC Pertide Statistics Peptide I Create Accession Peptide Unique peptide QP9457 4 4 Pertide Unique peptide QP9457 4 4 Pertide QP957 4 4 Per	Review Peak Peptides Identify QC Metrics Identified Picking Statistics Peptides QC Metrics Identified Peptides Unique peptides Confidence score Q 9457 4 4 31 PP37219 3 3 2239 Q 595W63 2 1 111 P 43066 3 3 2438 Q 98627 7 7 1 237 Q 728E 3 3 No tags to assign Q 41Nc2(+1) 9 9 New tag Q 728E 3 3 Wo tags to assign Q 41Nc2(+1) 9 9 New tag Q 42828 4 3 Edit tags Q 42828 4 3 Edit tags Q 42828 4 3 Edit tags Q 42828 4 4 26 C 44YN1 4 2 254 Q 44 4 26 Q 591Q6 1 1 8.066 Q 99409 25 25 260 m Selected protein: Isocitrate Iyase GN=ICL1 View peptide measurements Page 115	Review Peak Peptides Identify Petides QC Metrics Identifications Period Petides Confidence score Anova (p) Q 9457 4 4 31 136E-06 Q 9457 4 4 3 1 136E-06 Q 9457 7 7 7 1 257 1457 1457 1457 1457 1457 1457 1457 14	Review Pesk Picing Peptides Identify Peptides Refine QC Metrics Refine Beneficies Review Period Period No filter applied Create Search Peptides Onder Statistics Period No filter applied Create Search Peptides Confidence score Anova (p) q Value Q P457 4 4 31 138-06 6046-05 Q P97219 3 22.9 21.82-06 1.266-05 1.046-05 Q P457 7 Co Page 1.266-05 1.046-05 Q Q728E8 3 Quick Tags Anov Page 1.266-05 2.146-05 2.146-05 2.146-05 Q Q728E8 3 Quick Tags Anov Page Anov Page Anov Q C4YNS2 2 2 Add to Clip Galley Seque Page Anov Q C4VNI1 2 25.4 1.326-05 2.146-05 Page Q C4VNI1 2 2.5 1.46-05 2.146-05 Page Q S98/Q6 1 8.06 1.59-05 2.146-05	Review Pesk Picing Peptides Identify Peptide QC Metrics Refine Identification Review Profest Proteins Proteins Statistics Image: Statistics Peptide Center Search Peptide Peptid	Review Peak Picking Peptides Statistics Identify peptide Refine QC Metric Refine Interface Review Proteins Proteins Proteins Report Image: Comparison of the statistics Review Period Image: Comparison of the statistics Review Proteins Proteins <t< td=""><td>Review Pesk Picing Peptide Statistics Identify Peptide QC Metrics Refine Review Proteins Proteins Proteins Report Image: Construction of the second of the se</td><td>Pricing Statistics Perticities QC Metrics Identifications Proteins Statistics Report Accession Peptide Unique peptides Confidence score Anova (p) Q Value Tag Max fold change Highest Mean Lowest Mean Q 9P457 4 4 31 1366-06 A73E-06 1.95 B A Q 9P457 4 4 31 1366-06 141 C A Q 9P457 4 4 31 22.9 2.13E-06 1.04E-05 1.35 A C Q 9P457 3 2.2.9 2.13E-06 1.04E-05 1.78 B A Q 959063 2 1 11.1 2.65E-06 1.04E-05 1.78 B A Q 97288 3 3 Quick Tags 1.22E-05 3.28 C A Q 47289 3 3 Quick Tags Anova p-value B A Q 4440 Chip Galley 2 2.65 1.4E-05 2.77 B A Q 444 2</td></t<>	Review Pesk Picing Peptide Statistics Identify Peptide QC Metrics Refine Review Proteins Proteins Proteins Report Image: Construction of the second of the se	Pricing Statistics Perticities QC Metrics Identifications Proteins Statistics Report Accession Peptide Unique peptides Confidence score Anova (p) Q Value Tag Max fold change Highest Mean Lowest Mean Q 9P457 4 4 31 1366-06 A73E-06 1.95 B A Q 9P457 4 4 31 1366-06 141 C A Q 9P457 4 4 31 22.9 2.13E-06 1.04E-05 1.35 A C Q 9P457 3 2.2.9 2.13E-06 1.04E-05 1.78 B A Q 959063 2 1 11.1 2.65E-06 1.04E-05 1.78 B A Q 97288 3 3 Quick Tags 1.22E-05 3.28 C A Q 47289 3 3 Quick Tags Anova p-value B A Q 4440 Chip Galley 2 2.65 1.4E-05 2.77 B A Q 444 2

As an example, let us explore Isocitrate Lyase.

First filter the table to show only proteins showing a significant 2 fold or greater change.

File mport Data	Review Experiment Alignment Filtering Design Setup		eptide Ion Statistics	Identify Peptides QC	Refine Metrics Identificat		riew teins	Protein Statistics	Report			nonline A Waters Compa
					• •			-		SI ME	1.8	
Review Pro Jsing this scr Interest in you	oteins een, you can find the proteins of ur experiment.		e r applied s may be hidd	len Edit	Search		9					@ Hel
	quantitation options	Accession	Peptides	Unique peptides	Confidence score	Anova (p)	q Value	Tag 🔹	Max fold change	Highest Mean	Lowest Mean	Description
	not already done so, choose between and absolute quantitation, use of Hi-N,	Q9UVL1	3	3	26.5	5.87E-07	6.56E-06	4	2.44	A	С	Non-histone chrom
	rouping and more.	C4YL44	2	2	12.8	7.7E-07	6.56E-06	۹	2.19	В	Α	Extracellular matrix
	Protein options	P87219	3	3	22.9	2.13E-06	1.04E-05	۷	4.1	С	Α	Sorbose reductase
		Q9P8Q7		7			1.26E-05	4				Isocitrate lyase GN:
	a shortlist to review ble, sort and filter the proteins based	Q7Z8E8	3	3	21.8	4.42E-06	1.26E-05	4	3.28	С	Α	Cell surface hydrop
on their	measurements, to generate a shortlist	P42800	4	4	26	1.49E-05	2.14E-05	4	2.7	В	A	Inositol-3-phospha
	er review. How are the measurements calculated?	S C4YF25	1	1	6.57	0.000162	7.27E-05	4	2.26	A	В	Bud site selection p
-		C4YRH4	6	6	35.4	0.0011	0.000218	4	2.11	В	Α	NAD(P)H-depende
	he table by a given value, simply click ant column header.	C4YE92	2	2	13.4	0.00125	0.000234	4	2.65	с	Α	6_7-dimethyl-8-rib
	the proteins	Q5ALX8	5	5	51.3	0.00205	0.000314	4	2.05	с	A	Adenine phosphori
	protein of interest, review its peptide	Q59U83	1	1	5.68	0.00278	0.000395	4	4.06	с	A	Ribonucleoside-dip
measure	ments and correlations:	Q9HFQ6	1	1	14.6	0.00406	0.000492	4	2.22	с	A	60S acidic ribosom
	View peptide measurements	Q59NN8	1	1	6.72	0.0122	0.00108		3.46	A	в	Hsp70 nucleotide e
You can	also double-click to review a protein.	Q59WG0	1	1	7	0.0146	0.00124		3.24	с	A	Hit family protein 1
By expor	lata for further processing ting your data to external tools, there's to your analysis.	Selected View peptide n		lsocitrate ly	"ase GN=ICL1	PE=3 S	V=1					÷
	Export to pathways tool			А				В			С	
	Export protein measurements	11.5 T										
	Export peptide measurements	P 11.0										
	Export peptide ion measurements	ile 10.5) • •1			141	
	export pupple for measurements	ArcSinh Normalised 10.2 10.0 6.6 6 0.0 10.0 10.0 10.0 10.0 10.0 10.0 10.0		MAN .								
xperiment		4										
eview your o	lata from a different perspective:											

The table indicates that this protein is most highly expressed in Condition B, 2.01 fold over the lowest condition (A).

To view the corresponding peptide measurements for the current protein either double click on the protein in the table or use the **View peptide measurements** beside table and ensure that Show **Peptides** is selected.

P Progenesis QI.p Tutorial HDMSe - Progenesis QI for pro File Review Experiment		• w Peak Peptide Ion		Identify		Refine	Review	Protein					
Import Data Alignment Filtering Design Setup		king Statistics		Peptides	QC Metrics	Identification		Statistics	Report			no	nlinea
$\bullet \bullet \bullet \bullet$								•	0.1			A Wa	aters Compan
O Return to list of proteins O Previous protein	0 🕆 N	ext protein											🕜 Help
Review selected protein	Acc	ession: <u>Q9P8Q7</u>	0								🖘 No fi	lter applied 🦳	
teview the selected protein's identified peptides and alidate their expression patterns.	Des	cription: Isocitrate	lyas	e GN=I	CL1 PE=3 SV=1						Y		Create
Choose the level of detail	Σ	Identifier	Ions	s Score	Correlation	Anova (p)	Max Fold Change	Highest Mean	Lowest Mean	Tag	Abundance	Neutral Mass	Retention
View the properties and expression profiles of	٠	53.57_1729.8822n	1	7.423	0.957	0.000368	1.52	В	A		2.817E+04	1729.8822	53.566
either peptides or individual peptide ions: Show: Peptides Peptide ions	•	64.49_1503.8215n		7.388	0.934	2.92E-05					1.807E+04	1503.8215	64.486
	•	63.34_1322.6874n		7.247	0.971	2.61E-06					1.379E+04	1322.6874	63.343
Tip: you can also double-click a peptide to select and view its component ions.	0	53.57_1729.8549n	1	7.423	0.991	0.000386	7.4	В	Α	-	3626	1729.8549	53.566
Compare expression profiles	0	38.07_1302.7206n	1	6.440	0.989	5.47E-06	2.08	В	А	-	5533	1302.7206	38.068
Select peptides in the table to show their	0	38.09_1302.7213n	1	6.440	0.878	0.0814	2.08	В	А		634.5	1302.7213	38.086
expression profiles in the chart below.	0	38.29_1302.6459n	1	6.440	0.943	0.00224	1.32	В	Α		4070	1302.6459	38.291
Select all peptides	0	47.52_1312.7092n	1	6.960	0.970	1.06E-05	2.9	В	Α	-	7300	1312.7092	47.517
Correlation values for the expression profiles can	0	34.10_1489.7484n	1	7.208	0.991	3.44E-05	3.21	В	A	-	8931	1489.7484	34.097
also be seen in the table.	0	46.27_1696.8563n	1	5.650	0.016	0.0126	1.26	Α	с		3183	1696.8563	46.27
Resolve any quantitative outliers	-												
Tag any peptide ions whose expression profile is an outlier for this protein.					St.	andardised Ex	pression Profiles		-	1			
Learn about tagging and filtering							pression romes			J			
You can then review their identifications in more		A				В			C				
detail at the <u>Refine Identifications</u> step.	2												
	e e	0					A state	-	-				
	pur					1	//*			-			
	de la constante de	1										e e e e e e e e e e e e e e e e e e e	
	and see											0	
	Stinda	î 🛛 🛍	_	_	:								
xperiment design Review your data from a different perspective:	- 1	1			0								
		0 I											

The solid icon in Σ column indicates that the peptide contributes to protein measurements.

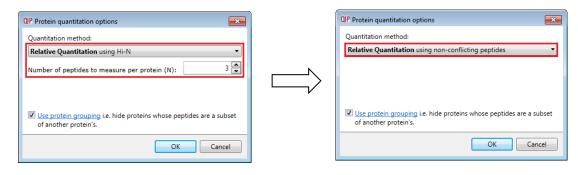
Note: a correlation score (between -1 and 1) for each peptide is available to facilitate the validation of peptide expression.

You can control what peptides are used in Protein Quantitation by using the **Protein options...** at the protein level of **Review Proteins**

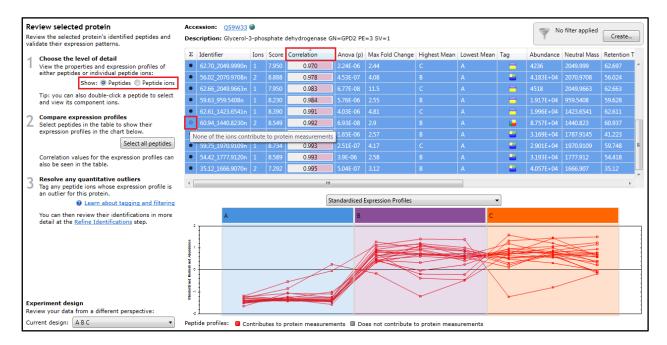
Usin	riew Proteins Ig this screen, you can find the pro rest in your experiment.	oteins of	Tag filter proteins m	applied ay be hidd	en Edit
1	Set the quantitation options		Accession	Peptides	Unique peptides
	If you've not already done so, cho relative and absolute quantitation		C4YL44	2	2 :
	protein grouping and more.	, use of Hi-N,	P87219	3	3
	Pr	otein options	🔮 Q9P8Q7	7	7
			Q7Z8E8	3	3
2	Create a shortlist to review	atoing based	P42800	4	4
	In the table, sort and <u>filter the pro-</u> on their measurements, to genera		S C4YF25	1	1 0
	for further review.	ante en la vieta da	C4YRH4	6	6
	How are the measurement	ents calculated?			

Note: the default quantitation method is based on the Relative Quantitation using Hi-N, in this case n=3 so the top 3 most abundant peptides are used to determine the relative abundance for each protein. Where there are multiple charge states the combined abundance of the charge states is used.

Changing to **Relative Quantitation using non conflicting** peptides will utilise all non conflicting peptides in the abundance calculation. (as shown below)



Note: doing this will update the peptide table to show open symbols against conflicting peptides which will not contribute to the quantitation of the protein.



In this example if you view the data at the level of the peptide ions then the two open symbols correspond to the 2 charge states of the conflicting peptide ion

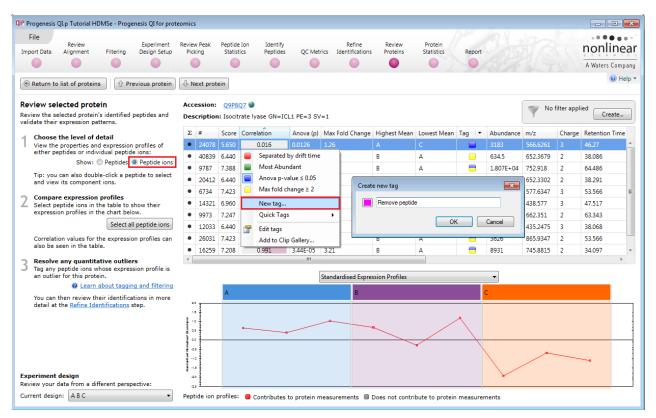
Review selected protein Review the selected protein's identified peptides and		ssion: Q59						No filter applied Create	_
validate their expression patterns.	Desci	ription: Gly	cerol-3-pl	hosphate dehydrogena		Greate			
Choose the level of detail	dance	m/z	Charge	Retention Time (mins)	Mass error (ppm)	Drift time (ms)	Peptide Sequence	Modifications	
View the properties and expression profiles of	E+04	596.3515			10.56	3.66	S LLSTSITDTLK		ŀ
either peptides or individual peptide ions:	E+04	691.3333	3	55.989	12.24	3.93	STFTEESAGVADLITTCSGGR	[16] Carbamidomethyl C	
Show: O Peptides O Peptide ions		481.2813	3	60.937	2.25	2.83	VIEDVVGASIAGALK		
Tip: you can also double-click a peptide to select and view its component ions.	E+04	721.4193	2	60.937	3.56	4.48	VIEDVVGASIAGALK		
	-								Þ

Returning to Isocitrate Lyase and the Peptides view of the table

Rev	riew selected protein iew the selected protein's identified peptides and date their expression patterns.		ession: <u>Q9P8Q7</u>		GN=IC	L1 PE=3 SV=1						V No	o filter applied	Create	
	Choose the level of detail	Σ	Identifier	Ions	Score	Correlation	Anova (p)	Max Fold Change	Highest Mean	Lowest Mean	Tag	Abundance	Neutral Mass	Retention	F I
1	View the properties and expression profiles of	٠	46.27_1696.8563n	1	5.650	0.016	0.0126	1.26	Α	С		3183	1696.8563	46.27	*
	either peptides or individual peptide ions: Show: Peptides Peptide ions	•	38.09_1302.7213n	1	6.440	0.878	0.0814	2.08	В	А		634.5	1302.7213	38.086	
		•	64.49_1503.8215n	1	7.388	0.934	2.92E-05	2.72	В	A	8	1.807E+04	1503.8215	64.486	
	Tip: you can also double-click a peptide to select and view its component ions.	•	38.29_1302.6459n	1	6.440	0.943	0.00224					4070	1302.6459	38.291	-

If you order the Peptide table using the **Correlation** column then hold down the Ctrl key and then click on the first peptide (with the lowest correlation) you can remove them from the expression profiles.

As an example: the removal of a poorly correlated peptide from a protein requires you to first create a tag (at the peptide ion level for the corresponding peptide ions of the peptide you wish to remove (see below).



Once the tag is created, return to the peptide level, to identify additional, poorly correlated peptides. For each additional peptide, double click on it and then right click on the corresponding peptide ion to select and add the **Remove peptide** tag, the tag appears in the tag column.

	view selected protein	Acc	ession:	<u>Q9P8</u>	Q7 🚳										Se Not	filter appl	
	iew the selected protein's identified peptides and date their expression patterns.	Des	cription	: Isocit	rate lyase GN=IC	L1 PE=	3 S\	V=1							1		Create
4	Choose the level of detail	Σ	#	Score	Correlation	Anov	a (p)	Max Fold Change	Highes	t Mean	Lowest Mean	Tag	•	Abundance	m/z	Charge	Retention Time (m
	View the properties and expression profiles of	٠	24078	5.650	0.016	0.012	5	1.26	Α		С			3183	566.6261	3	46.27
	either peptides or individual peptide ions:	•	40839	6.440	0.878	0.001		200	•		А	-		634.5	652.3679	2	38.086
	Show: 🔘 Peptides 💿 Peptide ions	٠	9787	7.388	0.934	2		eparated by drift time	•		A			1.807E+04	752.918	2	64.486
	Tip: you can also double-click a peptide to select and view its component ions.	•	20412	6.440	0.943	0		lost Abundant			Α			4070	652.3302	2	38.291
		•	6734	7.423	0.957	0		nova p-value ≤ 0.05 lax fold change ≥ 2			Α			2.817E+04	577.6347	3	53.566
2	Compare expression profiles Select peptide ions in the table to show their	•	14321	6.960	0.970	1	_	emove peptide			Α)	7300	438.577	3	47.517
	expression profiles in the chart below.	•	9973	7.247	0.971	2	-				Α	-)	1.379E+04	662.351	2	63.343
	Select all peptide ions	•	12033	6.440	0.989	5		ew tag			Α	-)	5533	435.2475	3	38.068
	Correlation values for the expression profiles can	•	26031	7.423	0.991	0	Q	uick Tags			Α	-)	3626	865.9347	2	53.566
	also be seen in the table.		16259	7.208	0.991	3 🚰	Ed	dit tags			Α	-)	8931	745.8815	2	34.097
2	Resolve any quantitative outliers	4			1		Ac	dd to Clip Gallery									+

Now at the Peptides level, set up a Tag Filter to display only the poorly correlated peptide ions in this example 2 (with the **Remove peptide** tag).

File	Review		Experiment	Review	Peak Peptide I	n	Identify		Refine	Review	Protein					
import Data	Alignment	Filtering	Design Setup	Picki			Peptides	QC Metrics	Identification	s Proteins	Statistics	Report			nonli	nea
				9								0/3			A Waters	Compa
0																🕡 Hel
	o list of proteins		evious protein		ext protein											e nei
Review se Review the s	elected proteins elected proteins r expression pati	in s identified		Acce	ext protein ession: 09P80 cription: Isocitra	-	e GN=IC	L1 PE=3 SV=1						g filter applie otides may be	ed The second	lit
Review se Review the s ralidate their	lected protein's	in s identified terns.		Acce Desc	ssion: <u>Q9P8Q</u>	-		L1 PE=3 SV=1	Anova (p)	Max Fold Change	Highest Mean	Lowest Mean	T per	otides may be	ed hidden Ed	lit
Review se teview the s ralidate their Choose View the	lected protein's elected protein's r expression pat	in s identified terns. etail expression	peptides and	Acce Desc	ession: <u>Q9P8Q</u>	te lyase Ions			Anova (p) 0.0126	Max Fold Change 1.26	Highest Mean A	Lowest Mean C	T per	otides may be	ed hidden Ed	lit

Then click on Refine Identifications on the workflow.

Select the peptides showing in the left hand table and then right click on one of the highlighted rows in the right hand table and click **Delete selected peptide(s)**.

File mport Data	Review Alignment	Filtering	Experiment Design Setup	Review Picki		Peptide Ior Statistics		entify ptides	QC Metri	ics Id	Refine entifications	Revie Prote		Report				nonline
				•				•						•				A Waters Compa
efine Ide	ntifications			Bato	h deleti	on criteria												
	de identificatio ults, you can i						Score [ess than	•				Seq	uence Length	less than	•		
	a set of dele						Hits	ess than	•					Charge	less than	•		
values fo	atch deletion o or a set of ider						Mass [ess than	•					Sequence	contains	-		
delete.				Abs	olute m	ass error (ppm) [ess than	•					Accession	contains	•		
	the unwanted e the identifica						m/z [ess than	•					Description	contains	-		
either:	ete Matching S	earch Pecult	s to delete			Retention	Time [ess than	•					Modifications	contains	•		
the • Dele	highlighted ID ete Non-match ete the IDs tha	s ing Search R	tesults, to										Delete matching s	earch results	Delete non	-matching sear	ch results	Reset the criteria
Bacat t	he criteria to	ctart again			#	Score	Hits	m/z	RT(mins) Charg	e Mass	Mass err	Sequence	Accessio	n Mor	difications		
To specif	fy another bat	ch of identific	cations to		24029	6.08	2	449.74	53.39	2	897.46	-2.89	GSFFAELK	C4YTC6				cted RNA polyme
delete, o step 1 a	lick Reset the bove.	Criteria and	then return to		24043	6.71	3	752.86	70.54	2	1503.7(SGNYFFL	Q5AI20				ling cassette sub-f
				v	24055 24055	6.63 6.63	3 3	790.71 790.71	36.73 36.73	3 3	2369.09 2369.09		GIAYQQDA GIAYOODA	Q9HG19 P43076				ated protein 1 OS: onsive protein 1 GN
	ilter applied de ions may be	hiddon	Edit		24055	6.63	3	790.71	36.73	3	2369.05		GIATQQDA	-			- C - C - C - C - C - C - C - C - C - C	V=PHR1 PE=4 SV=
pepuc	te ions may be	nidden (24055	8.19	6	1052.54		2	2103.06		VSILSDPO	Q9P8J8 Q9P8J8 Q9P8J8 Q9P8J8				nal purine biosynt
Total	Hits m/z	RT (min Ch	arge Tag 💌		24078	5.65	3	566.63	46.27	3			<u> </u>	09P807				lyase GN=ICL1 PE
			-		24099	7.24	4	770.44	46.41	2	Delet	e selected	peptide(s)	C4YRA2				c translation initia
083! 2	652.367	38.09 2	-	1	24103	6.07	2	510.28	55.07	2	1018.5!	7.12	EVALDFIGR	Q5A8Y5			DNA-dire	cted RNA polyme
					24138	6.43	8	878.09	60.17	3	2631.24	5.30	DCPVHLSY	Q5AK86	[2] Carba	midomethyl C	Arginase	GN=CAR1 PE=3 S
				1	24143	5.52	2	553.62	60.09	3	1657.83	17.55	🎯 GTTISSTGA	O13434			Phospho	enolpyruvate carb
				1	24143	5.52	3	553.62	60.09	3	1657.83	17.55	🎯 GTTISSTGA	C4YNC2			Phospho	enolpyruvate carb
					24149	7.22	9	776.41	41.81	2	1550.81	2.79	🎯 IAGAALDV	Q5A3K7			D-3-pho:	phoglycerate deh
				1	24166	7.61	9	993.20	56.80	3	2976.57	4.77	GLSVDAVS	O94039			Transket	olase 1 GN=TKT1 P
				1	24187	5.46	3	470.50	45.80	4	1877.9(3.68	IFITDNDSIE	Q5A2X3			Ubiquitin	-activating enzym
				1	24195	6.49	9	777.87	58.88	2	1553.72	-72.92	AGATPEVQ	C4YF03			Phenylala	anyl-tRNA syntheti
					24250	6.03	5	650.81	49.55	2	1299.61	5.82	IFDLDGTD	Q5AEF2			Protein t	ansport protein SE

Now return to **Review Proteins**, double click on Isocitrate Lyase (Q9P8Q7) and go to the **Peptides** view. Clear the filter, to view the remaining peptides, and then click **Select all peptides** for Isocitrate Lyase.

Note: the poorly correlated peptides are no longer present.

		eptide Ion	Identify		Refine	Review	Protein				r	online
nport Data Alignment Filtering Design Setup	Picking	Statistics	Peptides	QC Metrics	Identifications	Proteins	Statistics	Report			<u> </u>	Y N A M T I
Return to list of proteins	A Next protei					•		1	~~~~~			Waters Compa V Hel
eview selected protein wiew the selected protein's identified peptides and	Accession: Description:		se GN=I	CL1 PE=3 SV=1						N	o filter applied	Create
lidate their expression patterns.	Σ Identifier			Correlation	A	M. F.H.C	10.1.1.1.1		т		Neutral Mass	Retention Tir
Choose the level of detail View the properties and expression profiles of		02.6459n 1	ns Score 6.440	0.945		Max Fold Change	B	A Lowest Mean	Tag	4070	1302.6459	38.291
either peptides or individual peptide ions:		29.8822n 1	7.423	0.940		1.52	B	A		2.817E+04	1729.8822	53.566
Show: 💿 Peptides 🔘 Peptide ions		03.8215n 1	7.388	0.900		2.72	B			1.807E+04	1503.8215	64.486
Tip: you can also double-click a peptide to select and view its component ions.	• 47.52 13		6.960	0.985			B			7300	1312.7092	47.517
	-	29.8549n 1		0.989	0.000386		B				1729.8549	53.566
Compare expression profiles Select peptides in the table to show their	• 38.07_13	02.7206n 1	6.440	0.990	5.47E-06		В				1302.7206	38.068
expression profiles in the chart below.	• 63.34_13		7.247	0.992	2.61E-06				-	1.379E+04	1322.6874	63.343
Select all peptides	• 34.10_14	89.7484n 1	7.208	0.995	3.44E-05	3.21			8		1489.7484	34.097
Correlation values for the expression profiles can also be seen in the table.	•				m							
Resolve any quantitative outliers				ĺ	Standardised E	xpression Profiles			•			
Tag any peptide ions whose expression profile is an outlier for this protein.		A			В			c	:			
Learn about tagging and filtering	2.0	_										
You can then review their identifications in more detail at the <u>Refine Identifications</u> step.	1.5 aurepunge 0.5 0.5											
xperiment design	paripurpurg -1.0 -1.5	/ ///										2
eview your data from a different perspective:	-20	_										

Now return to the protein level by clicking on Return to list of proteins (top left).

Before creating tags for proteins containing modified peptides, reset the Quantitation method to **Relative Quantitation using Hi-N** using the **Protein options...**

Now right click on the Protein table and select **Modification** from the **Quick tags**.

P Protein quantitation options
Quantitation method:
Relative Quantitation using Hi-N
Number of peptides to measure per protein (N): 3
Use protein grouping i.e. hide proteins whose peptides are a subset of another protein's.
OK Cancel

Review Proteins Using this screen, you can find the proteins of interest in your experiment.	When the test of test	applied	Create	earch	Q			
 Set the quantitation options 	Accession	Peptid	es Unique peptides	Confidence score	Anova (p)	q Value	Tag 💌	Max fo
If you've not already done so, choose between relative and absolute quantitation,	🔇 Q9P457	Anov	ap-value ≤ 0.05	1	1.36E-06	8.73E-06	-	1.95
use of Hi-N, protein grouping and more.	P87219		old change ≥ 2	2.9	2.13E-06	1.04E-05	•	4.1
Protein options	Q59W63			.1.1	2.65E-06	1.04E-05	•	1.35
	P43066	New		11.8	2.84E-06	1.04E-05	•	1.78
7 Create a shortlist to review	Q9P8Q7	Quicl	: Tags I	Anova p-v	/alue	05		2.01
In the table, sort and <u>filter the proteins</u> based on their measurements, to generate a	Q7Z8E8	P Edit t	ags	Max fold o	change)5	•	3.28
shortlist for further review.	C4YNC2 (Add t	o Clip Gallery	Modificati	ion)5		1.51
<u>How are the measurements calculated?</u>	C4YS49	3	3	2 Sequence)5	•	1.18
To sort the table by a given value, simply click the relevant column header.	Q5AEB8	4	3	Peptide ta	gs contain	2.1-+05	•	1.41

To find those proteins containing peptides with Carbamidomethylated cysteine and Oxidated methionine residues create Quick tags for each modification as shown below.

OP New Quick Tag	QIP New Quick Tag
Where any peptide of a protein has Modification with: Carbamidomethyl C	Where any peptide of a protein has Modification with: Oxidation M
Can I use wildcards?	Can I use wildcards?
Apply the following tag:	Apply the following tag:
Modification with Carbamidomethyl C	Modification with Oxidation M
Create tag Cancel	Create tag Cancel

To reduce the table to displaying only these proteins with modified peptides (on cysteines and or methionines) use a tag filter to focus on these proteins by placing the appropriate tags in the **Show proteins that have at least one of these tags:**

vailable tags:	Show proteins that have all of these tags:
 Anova p-value ≤ 0.05 (375 proteins) Max fold change ≥ 2 (15 proteins) 	Show proteins that have at least one of these tags:
	Modification with Carbamidomethyl C (208 protein Modification with Oxidation M (26 proteins)
	Hide proteins that have any of these tags:

The proteins table will now only display those proteins containing the modified peptides.

File mport D	Review Jata Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Statist		Identify Peptides QC Me	Refine trics Identificat	Review ons Protein:			nonlinea A Waters Compar
lsing th	v Proteins is screen, you can in your experime		oteins of		g filter ap oteins may		n Edit	Search	م			 Waters company Help
	the quantitatio			Anova (p)	q Value	Tag 💌	Max fold change	Highest Mean	Lowest Mean	Description		
	ou've not already ative and absolute			0.000465	0.000134	4	1.26	Α	С	Ribosome biogenesis protein YTM1 GN	V=YTM1 PE=3 SV=1	
pro	tein grouping and	more.		0.000481	0.000137	•	1.24	В	A	UTP-glucose-1-phosphate uridylyltrans	sferase GN=UGP1 PE=4 SV=1	
		Pr	rotein options	0.000501	0.000137		1.76	С	A	Pyruvate decarboxylase GN=PDC11 PE	=3 SV=1	
				0.000503	0.000137		Anova p-value ≤ (0.05		Sti1 GN=STI1 PE=4 SV=1		
	eate a shortlist t the table, sort and		roteins based	0.000521 0.000139 🛛 🕘 Modification with Carbam				iyl C	Threonine aldolase GN=GLY1 PE=3 SV			
	their measuremen further review.	nts, to gener	ate a shortlist	0.000523	0.000523 0.000139 🛛 🍚 Mo			Modification with Oxidation M		Fatty acid synthase alpha subunit reduc	ctase GN=CAWG_02796 PE=4 SV=	1
How are the measurements calculate		ents calculated?	0.000541	0.000143		1.42	۸	C	60S ribosomal protein L10 GN=RPL10	PE=4 SV=1		
То	sort the table by a	a given value	, simply click	0.000559	0.000146		1.4	В	Α	Aldehyde dehydrogenase_ mitochondr	ial GN=ALD5 PE=3 SV=1	
the	relevant column	header.		0.000591	0.000153	-	1.19	В	A	Tyrosyl-tRNA synthetase GN=TYS1 PE=	=4 SV=1	
Re	view the proteir	IS		0.000719	0.000174	-	1.31	Α	С	Inorganic phosphate transporter PHO8	4 GN=CAWG_00289 PE=4 SV=1	
	each protein of in asurements and o		ew its peptide	0.000724	0.000174	-	1.15	В	A	Adenylyl-sulfate kinase GN=MET14 PE:	=3 SV=1	
ine		w peptide m	anguramenta	0.000738	0.000174		1.45	В	Α	Peroxiredoxin TSA1 GN=TSA1 PE=2 SV	'=1	
					0.000174	-	1.4	В	Α	Heat shock protein SSA2 GN=SSA2 PE:	=1 SV=3	
You	i can also double-	click to revie	w a protein.	*						III		•
By	port data for fur exporting your da limit to your analy	ta to externa		Selector View pept			yruvate deca	arboxylase	GN=PDC1		с	
		xport to path ort protein m	nways tool neasurements	14.0 ·			A			В	C	
			measurements measurements	N 13.0 ·			P			a∰t.	I ∎ I	
				Arcs: 12.0								
	nent design				4							
	your data from a	airrerent per	spective:								Casti	on Complete
	design: ABC		•		his sector	the states	layed: 217					on complete

Note: hold the cursor over the tags for a description of the protein's current tags.

Note: the Sequence Quick tag can be used to locate Proteins containing peptides with specific motifs.

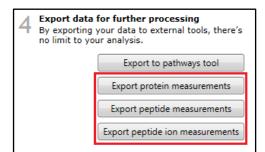
Stage 13: Exporting Protein Data

Data can be exported in a csv file format. You can either export the **protein**, **peptide or peptide ion measurements** using the options in the File Menu or use the buttons under Step 4 both available at the **Review Proteins** stage.

As an example of Data export use the Tag filtered set from the previous section for only proteins that have Oxidised Methionine residues.

First set the tag filter as shown below. Then select **Export Protein Measurements**.

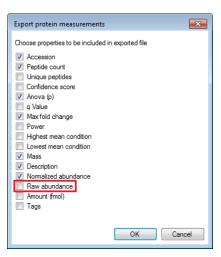
QIP Filter the proteins	×
Create a filter Show or hide proteins based on a selection of the create the filter. For more guidance, please see th	
Available tags:	Show proteins that have all of these tags:
 Anova p-value ≤ 0.05 (377 proteins) Modification with Carbamidomethyl C (207 p Max fold change ≥ 2 (15 proteins) 	Modification with ox (25 proteins) Show proteins that have at least one of these tags: Hide proteins that have any of these tags:
«	
Clear the filter	OK Cancel

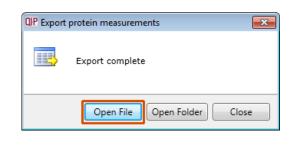


	rogenesis QI.p Tutorial HDMSe - Progenesis QI for prote File
	Save
	Close
	Export peptide ion measurements
	Export peptide measurements
	Export protein measurements
	Export to pathways tool
	Import additional protein data
	Import protein accessions as tag
	Export mzIdentML for PRIDE submission
	Experiment properties
	Show Clip Gallery
×	Exit

The Export Protein Measurements dialog opens. Select the required fields and click OK. **Save** the file and then open the exported data file using the dialog that opens.

Note: if you have performed Absolute Quantification then the 'Amount' field will be available.





Excel will open displaying the exported protein measurements

						Normalize	d abundar	nce						
						A			В			С		
Accession	Peptide count	Anova (p)	Max fold change	Mass	Description	A_01	A_02	A_03	B_01	B_02	B_03	C_01	C_02	C_03
Q59WG3	19	0.000286	1.393265562	91276.19	Cell division control protein 48 GN=CDC48 PE=3 SV=1	6.04165	6.104069	6.382303	7.810237	8.469195	8.124259	9.235389	8.411273	8.167793
Q5A1E2	19	0.000501	1.757985787	62783.45	Pyruvate decarboxylase GN=PDC11 PE=3 SV=1	30.47532	34.81616	35.18093	61.06671	57.59173	54.2401	64.75432	61.17964	50.6951
P46587	34	0.00075	1.395119219	70241.81	Heat shock protein SSA2 GN=SSA2 PE=1 SV=3	26.34676	27.96146	28.2539	36.62435	40.75456	37.80508	33.07268	33.9658	30.17681
Q9Y725	5	0.002606	1.242929018	39469.74	Mannose-1-phosphate guanyltransferase 1 OS=Candic	5.923814	6.143487	6.088348	5.100299	4.794784	4.712066	5.066129	5.133183	4.493118
Q59Z65	5	0.003284	1.239495422	31564.35	Proteasome component PRE2 GN=PRE2 PE=3 SV=1	2.14134	2.00107	1.959733	2.456694	2.641729	2.465155	2.293807	2.248603	2.465606
Q5ADM7	20	0.004259	1.785445695	35947.04	Glyceraldehyde 3-phosphate dehydrogenase GN=TDH	28.27132	37.47768	35.05756	66.21397	52.08768	50.68558	65.07962	63.1759	51.72912
Q59LW3	14	0.007171	1.929316718	187412.7	DNA-directed RNA polymerase GN=RPA190 PE=3 SV=1	4.219746	3.771511	3.794856	6.538796	4.287708	4.245464	8.246794	7.903809	6.588543
C4YTC6	10	0.00903	1.22209187	132058.3	DNA-directed RNA polymerase I 135 kDa polypeptide	9.059609	10.47197	9.842404	10.69975	11.48251	11.08544	9.237917	9.320345	8.663667
Q59N00	15	0.009063	1.182776974	27285.6	40S ribosomal protein S3 GN=RPS3 PE=4 SV=1	30.95634	32.97579	31.92331	30.09772	32.01223	30.41159	27.94132	28.06409	25.03729
C4YSV1	6	0.020115	1.197574763	15842.55	60S ribosomal protein L25 GN=CAWG_05170 PE=4 SV=1	19.09519	21.06827	20.28896	21.3714	20.04349	20.17272	17.93076	18.02852	15.46766
C4YQM1	4	0.022335	1.124601067	15995.81	40S ribosomal protein S15 GN=CAWG_02780 PE=4 SV=1	7.402242	7.74925	7.425526	7.501278	7.528884	7.452533	6.944223	6.957132	6.174225
P30575	18	0.026576	1.713187284	47231.58	Enolase 1 GN=ENO1 PE=1 SV=1	21.86692	29.96505	28.59235	51.95777	35.8221	35.52757	50.63594	49.372	37.77399
C4YHD6	7	0.031059	1.083204829	76870.01	NADPH-cytochrome P450 reductase GN=CAWG_03482	4.676369	5.02495	4.740551	5.020557	4.936881	4.881926	4.561702	4.682273	4.455524
C4YTC4	11	0.035844	1.174491751	91821.43	Glutaminyl-tRNA synthetase GN=CAWG_05411 PE=4 SV	9.448774	10.68459	9.762518	9.3306	8.400595	8.364039	8.782928	8.725951	7.945433
C4YRA2	9	0.041867	1.150593676	59119.25	Eukaryotic translation initiation factor 2 gamma subun	4.030048	4.073439	4.109665	5.053444	4.451311	4.54762	4.49272	4.689095	4.194535
P41797	27	0.056709	1.244772849	70495.2	Heat shock protein SSA1 GN=SSA1 PE=1 SV=2	15.05905	11.85971	11.42823	15.36704	16.33804	16.0282	13.4975	13.78449	12.17764
Q5A516	12	0.059216	1.179345683	32926.06	ADP_ATP carrier protein GN=PET9 PE=3 SV=1	30.36237	34.1933	32.87809	34.50778	35.19443	33.92876	30.93623	30.89512	26.04023
Q5ABS1	7	0.06238	1.069201884	14423.79	Ubiquinol-cytochrome c reductase complex 14 kDa pro	7.27818	7.431786	7.889224	8.234258	7.998214	7.930625	7.727017	7.876603	7.862713
Q5A900	9	0.078281	1.248516734	26987.34	40S ribosomal protein S2 GN=RPS21 PE=3 SV=1	15.07801	16.72155	15.13405	14.1835	12.10492	11.30308	15.55814	15.47995	12.76609
Q59M82	19	0.08783	1.124167619	190630.7	Clathrin heavy chain GN=CHC1 PE=4 SV=1	9.715146	10.26357	10.03633	10.34802	10.91702	9.966933	9.749938	9.565339	8.467036
C4YR46	38	0.099847	1.121176792	117623.1	Elongation factor 3 GN=CAWG_04545 PE=4 SV=1	23.97338	26.44841	25.42621	27.59592	29.83916	27.60393	26.99834	26.37865	23.40255
Q5ACQ0	11	0.137185	1.118347026	77833.41	Long-chain-fatty-acid-CoA ligase 4 GN=FAA4 PE=4 SV=	9.58484	10.05479	9.555411	9.596871	8.595828	8.515559	8.989106	9.185083	7.931336
P16017	19	0.234967	1.194311094	50468.03	Elongation factor 1-alpha GN=TEF1 PE=1 SV=1	68.59949	67.33289	65.09681	70.54589	49.78468	48.19559	69.49089	73.01285	58.76892
Q59ZX4	14	0.314524	1.114106362	39269.36	60S ribosomal protein L4-B GN=RPL4B PE=4 SV=1	37.76197	40.11539	38.34233	39.72134	36.06694	34.58901	37.5628	36.55015	30.20356

Note: where there are multiple group members the other accession numbers are also exported.

At each stage in the Work flow there are a number of Export and Import options available from the **File** Menu. This includes the option to **Import Additional Protein Data** which can be used to increase the Protein metadata and also be used to sort the existing tabular data.

QIP P	rogenesis QI.p Tutorial HDMSe - Progenesis QI for prot	e
	File	_
	Save	nics
	Close	Pe
	Export peptide ion measurements	
	Export peptide measurements	
	Export protein measurements	Acc
	Export to pathways tool	3
	Import additional protein data	0
	Import protein accessions as tag	
	Export mzIdentML for PRIDE submission	3 3
	Experiment properties	3
	Show Clip Gallery	
×	Exit	

Exporting Protein Data to Pathways Tool(s)

Using Progenesis, you can export protein lists to pathway analysis tools to help you understand your data in a wider biological context.

Currently Progenesis QI for proteomics supports the export to:

IMPaLA: which aggregates and queries many other pathway analysis tools including KEGG and Reactome

PANTHER: classifies proteins for high-throughput analysis.

MetaCore: MetaCore is an integrated software suite for functional

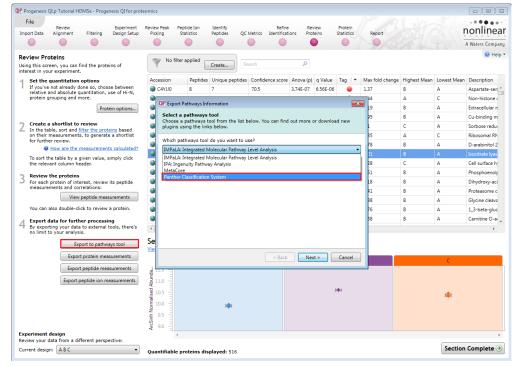
analysis of experimental data. MetaCore is based on a curated database of human protein-protein, protein-DNA interactions, transcription factors, signaling and metabolic pathways, disease and toxicity, and the effects of bioactive molecules.

Ingenuity Pathways Analysis (IPA): Explores experimental data by identifying relationships, mechanisms, functions, and pathways of relevance.

Note: Plugins for these tools are provided as standard.

Note: Access to IPA is dependent on having a local licence for IPA. Details of exporting data to IPA are described in Appendix 9 (page 100)

As an example of exporting to a Pathways tool first use the tag filtering to 'focus' on the set to export. Then click **Export to pathways tool**, select **Panther** and then the test to be performed.



Select either Statistical over-representation test or Statistical enrichment test.

Make sure the **Open Panther in my browser** is ticked and then click **Export proteins to file.** Save file with appropriate name.

When Panther opens locate the file, select the appropriate organism (Candida albicans) and tick Statistical enrichment test.

QIP Export Pathways Information
Configure your export Choose which identifications to export and the type of analysis you want to perform.
Select the type of analysis to perform:
Statistical enrichment test 🔹
For statistical enrichment testing, choose two experimental conditions that you would like to compare.
Baseline: Comparison:
🗖 A 🔹 📢 🖪 B
If greater, gives a negative fold change. If greater, gives a positive fold change.
To perform the pathway analysis, save the protein data to a file and select that file for the Upload IDs option on the Panther search page.
Open Panther in my browser
< Back Export proteins to file Close

e About PANTHER Data	PANTHER Tools Workspac	e Downloads Help/Tutorial
NTHER14.1 is generated	from the 2018_04 release	of ReferenceProteome dataset
rch		
	Gene List Analysis	Browse Sequence Search CSNP Scoring Keyword Search
•		
Go	Please refer to our articl	e in Nature Protocols for detailed instructions on how to use this page.
ck links	Help Tips	1. Enter ids and or select file for batch upload. Else enter ids or select file or list from
e genome function	Steps: 1. Select list and	workspace for comparing to a reference list.
me statistics	list type to analyze	Enter IDs: Supported separate IDs by a space or comma
Version	> 2. Select Organism	Supported IDs separate IDs by a space or comma
to cite PANTHER	> 3. Select	Upload Choose File Panther test.txt
Recent publication	operation	File
ibing PANTHER		format Please login to be able to select lists from your workspace.
5		Select List ID List
HER User Interface		Type: Previously exported text search results
tes		Workspace list
for additional info.		 PANTHER Generic Mapping ID's from Reference Proteome Genome
		Organism for id list Absidia glauca (ABSGL)
sletter subscription		VCF File Flanking region 20 Kb 🔻
r your Email:		
Subscribe		2. Select organism.
		Caenorhabditis brigosae Candida albicans
E		Canis lupus familiaris Chlamydia trachomatis
PostgreSQL		Championa calebookii
		3. Select Analysis.
		Functional classification viewed in gene list
		Functional classification viewed in graphic charts Bar chart Pie chart
		Statistical overrepresentation test
		Statistical enrichment test
		PANTHER Pathways

Click submit.

Analysis results list is returned:

8		Unifying Biolo	SY Classification System	utorial	LOGIN RI	Egister cot	NTACT US			
	Home About PANTHER Data PANTHER Tools Workspace Downloads Help/Tutorial PANTHER14.1 is generated from the 2018 04 release of ReferenceProteome dataset									
		R PATHWA	-	ne dataset						
		st to: -Selec								
Dice	lav: 3	0 Vitama	per page <u>Refine Search</u>							
	· _		 Page <u>Refine Sector</u> 2 Number of mapped ids found 52 IDs not found (176) 							
clr	all	Pathway Accession	Mapped IDs	Pathway Name	Components	<u>Subfamilies</u>	Associated Sequence			
	1.	P00005	CANAL CGD=CAL0000192659 UniProtKB=042825	Angiogenesis	77	246	<u>1254</u>			
	2.	P00052	CANAL EnsemblGenome=CaO19.1760 UniProtKB=Q59XU5 CANAL CGD=CAL0000188340 UniProtKB=P28870 CANAL CGD=CAL0000196167 UniProtKB=Q59VR3	<u>TGF-beta signaling</u> pathway	20	<u>168</u>	<u>830</u>			
	3.	<u>P02738</u>	CANAL CGD=CAL0000188396 UniProtKB=Q5A0L0 CANAL CGD=CAL0000191707 UniProtKB=Q5AG68 CANAL CGD=CAL0000179678 UniProtKB=Q5A6P2 CANAL CGD=CAL0000191899 UniProtKB=Q5A6R2	<u>De novo purine</u> <u>biosynthesis</u>	23	<u>81</u>	<u>575</u>			
	4.	P00059	CANAL CGD=CAL0000191263 UniProtKB=P43063	p53_pathway	<u>70</u>	<u>107</u>	<u>591</u>			
	5.	P00048	CANAL EnsemblGenome=CaO19.1760 UniProtKB=Q59XU5	<u>PI3 kinase pathway</u>	<u>35</u>	<u>78</u>	425			
	6.	P05734	CANAL CGD=CAL0000181188 UniProtKB=Q59YF0	Synaptic vesicle trafficking	<u>12</u>	<u>32</u>	<u>164</u>			
	7.	<u>P00024</u>	CANAL CGD=CAL0000173961 UniProtKB=Q9P940 CANAL CGD=CAL000197744 UniProtKB=Q5ADM7 CANAL CGD=CAL0000185645 UniProtKB=P30575 CANAL CGD=CAL0000185566 UniProtKB=P83780 CANAL CGD=CAL0000184591 UniProtKB=P82612 CANAL CGD=CAL0000184891 UniProtKB=P42273	Glycolysis	<u>10</u>	<u>42</u>	<u>141</u>			
	8.	P02723	CANAL CGD=CAL0000192887 UniProtKB=Q5ALX8	Adenine and hypoxanthine salvage_pathway	<u>10</u>	<u>21</u>	<u>120</u>			
	9.	<u>P00044</u>	CANAL CGD=CAL0000181188 UniProtKB=Q59YF0	Nicotinic acetylcholine receptor signaling pathway	<u>13</u>	252	<u>962</u>			
	10.	P02762	CANAL CGD=CAL0000194226 UniProtKB=Q5A017 CANAL CGD=CAL0000197690 UniProtKB=P83780	<u>Pentose phosphate</u> <u>pathway</u>	<u>13</u>	20	<u>57</u>			
	11.	<u>P02721</u>	CANAL CGD=CAL0000187344 UniProtKB=P0C8K9	ATP synthesis	<u>12</u>	<u>18</u>	<u>64</u>			
	12.	P00016	CANAL CGD=CAL0000192659 UniProtKB=O42825	Cytoskeletal regulation by Rho GTPase	<u>20</u>	<u>174</u>	<u>824</u>			

Click to explore returned analysis.

Note: the success of a Pathways analysis is dependent on the organism under study being available to search using **Impala** and **Panther**.

Clear any applied tag filters then move to the next section/stage.

Stage 13: Exporting identifications for submission to PRIDE

If you are submitting your raw data and results to PRIDE using the **mzIdentML** Complete submission process (described in section 2A on the PRIDE site) then it is now possible to export the identification results as mzIdentML which can be checked in **Pride Inspector tool** as part of the submission process.

Note: this only supports identifications obtained from searches performed in Progenesis QI for proteomics version 4.0 or later, using the search methods Mascot or Ion Accounting.

Export Identifications is carried out at the Review Proteins stage of the workflow. Click on the **File** menu and select **Export mzldentML for PRIDE submission...**

A dialog will appear allowing you to include the protein sequence or not in your export. Choosing to export the sequence will allow the display of the coverage map in PRIDE Inspector.

Enter a file name. Progenesis will save two files the mzldentML as a .mzid file and also a corresponding .mgf file containing the spectra.

Open PRIDE Inspector and locate and select the mzid file to import.

PRIDE Inspector 2.5.2 Open Export Help		
Quick Start	Try Examples	
Open Ider	Select mzML/mzXML/mzid/PRIDE xml Files Look In: Progenesis QLp v4 HDMSe Tutorial QLp_Tutorial A_Loaded_HDMSe.Analysis	
Review Pr	HDMSe 2 fold Significant.DerivedFromSearch880843c2-3cd5-4413-87be-21797717403d.mgf ML HDMSe 2 fold Significant mzid Image: State	
		More examples
Feedback	Files of Type:	
🖂 Give Us Your Fe	edback	\cap
When use PRIDE Inspector	please cite:	

Click open.

You will then be asked if you want to load the corresponding spectrum files. Click yes and locate the .mgf file.

UP P	rogenesis QI.p Tutorial HDMSe - Progenesis QI for prote
	File
	Save
	Close
	Export peptide ion measurements
	Export peptide measurements
	Export protein measurements
	Export to pathways tool
	Import additional protein data
	Import protein accessions as tag
	Export mzIdentML for PRIDE submission
	Experiment properties Show Clip Gallery
×	Exit

Export mzIdentML for PRIDE submission
Choose properties to be included in exported file
☑ Protein sequence
OK Cancel

A dialog opens and allows you to **Add spectra files.** Add the corresponding .mgf file and click **Set**

S Load spectrum files				💽 🕄 Help
ADMSe 2 fold Sig	File Name	Size (M)	Туре	Remove
0	HDMSe 2 fold Significant.Deriv	44.0278	MGF	×
	Add spectra files			
			Cancel	Set

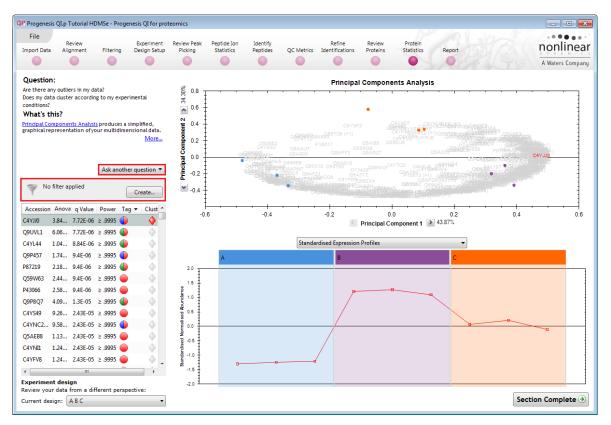
PRIDE Inspector will open allowing you to check the data.

RIDE Inspector 2.5.2								
en <u>E</u> xport <u>H</u> elp								
I.j Assays	Overview Protein Pepti	de Spectrum Sum	mary Charts					
🧪 HDMSe 2 fold Signific	III × Protein List				🛓 Update Protei	n Details 🛛 🍸 Decoy	/ Filter 🛛 🥜	
	Protein Group ID	Protein Group ID Protein			istinct Peptides	#PTMs		
	ProteinGroup_165_C4YP	V(C4YPV0	56	9		0		
	ProteinGroup_168_C4YF	JE <u>C4YFJ6</u>	97	9		3		
	ProteinGroup_293_Q5A9	4(<u>Q5A940</u>	26	5		0		
	ProteinGroup_458_Q5AJ	YE <mark>Q5AJY5</mark>	9	2		0		
	ProteinGroup_406_Q59P	Z4 <u>Q59PZ4</u>	11	3		1		
	ProteinGroup_251_C4YG	21 <u>C4YG28</u>	31	6		0		
	ProteinGroup_194_Q5AH	10 <u>Q5AH07</u>	45	8		2		
	ProteinGroup_515_C4YF	48 <u>C4YF48</u>	4	1		0		
	ProteinGroup_412_P536	96 <u>P53696</u>	10	3		1		
	ProteinGroup_241_Q9P8	ProteinGroup_241_Q9P844Q9P844		6		0		
	ProteinGroup_260_C4YL	ProteinGroup_260_C4YLN!C4YLN5		6		3		
	ProteinGroup_61_Q5ADU	ProteinGroup_61_Q5ADU2Q5ADU2		15		2		
	ProteinGroup_129_Q5AA	ProteinGroup_129_Q5AAU'Q5AAU7		10		2		
	Peptide LLNVEVPLR LLNVEVPLR	Ranking 1 1	Delta m/z Charg 0.0031 2 0.0031 2	526.8299 526.8299	Modifications Length 9 9	Start 173 173	Stop 181 181	
		1	0.0031 2	526.8299	9	173	181	
Assay Summary	LLNVEVPLR	1	0.0031 2	526,8299	9	173	181	
		1	0.0031 2	526.8299	9	173	181	
Spectra found			0.0001	500.0000		470	101	
Proteins found	Spectrum Fragmentation	n Table Sequence		_				
Protein Group found				Selecte	d 📕 PTM 📕 Fit	Fuzzy Fit	Overlap	
Peptides found	Accession: Q5A	H07						
Peplides Iound	45 peptides (45 i	matched, 8 distinct), 1	12/478 amino acids	(23.4% coverage)				
Mod: UNIMOD:4			THEOREM		DT HEDT OD HD	000000000000000000000000000000000000000	6.0	
Mod: UNIMOD:35	MLRSASRIS		_		PLVTPLGRHP	QKYSTPAPGF	60 12	
	GPTTFTEVL		2		PQHPAAHGVL	RLILELHGEE		
Imported MS/MS Annota	tions IVRSDPHVG				TNELVFALAV EREKLMEFYE	EK <mark>LLNVEVPL</mark> RVSGARLHTA	18 24	
	YFRPGGVSQ				RIWKDRTIGV	GVVSAEDALN	24 30	
	YSLSGVMLR		_		CYDRYLIRMA	GVVSAEDALN EFRQSLR IIF	30	
	OCINDIPEG		2		TK GYAVPOGE	TYTAIEAPKG	3 B 42	
	QCINDIPEG		PSKSLMKED		TRGIAVPQGE	THEATRA	44	

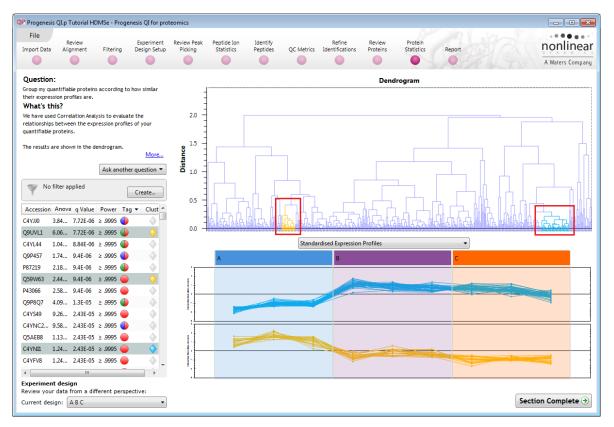
Once checked you can submit the results data to PRIDE, along with your raw data, as part of your submission process, following the guide lines provided on the PRIDE site.

Stage 14: Protein Statistics

Protein Statistics opens with a Principal Components Analysis (PCA) for all the proteins displayed.



The Multivariate Stats can now be applied to all or subsets of proteins as determined by the current Tag filters. Allowing you to identify similar paterns of expression using the Correlation Analysis. Click on 2 of the branches (holding the **Ctrl** key down) to see differing patterns of expression.



Now move to the **Report** section to report on Proteins and /or peptides.

Stage 15: Reporting

The **Report Design** stage allows you to select what views you want to include in a report based on the list of **currently selected proteins.**

Note: this facility is used to generate Html reports on a limited selection of Proteins in your data. Creating a report on all the data in your experiment can take a long time

QP Filter the proteins	
Create a filter Show or hide proteins based on a selection of their create the filter. For more guidance, please see the	
Available tags:	Show proteins that have all of these tags:
 Anova p-value ≤ 0.05 (375 proteins) Modification with Carbamidomethyl C (208 pro Modification with Oxidation M (26 proteins) 	Max fold change ≥ 2 (15 proteins) Show proteins that have at least one of these tags: Hide proteins that have any of these tags:
< III >	
Clear the filter	OK Cancel

As an example we will create a report for **only** the proteins showing a Max Fold change of greater than 2.

- 1. First reduce the proteins to report on by selecting the 'Max fold change ≥ 2' tag. In this example it reduces the number of proteins in the table to 15.
- 2. Expand the various Report Design options (by default they are all selected)
- 3. Un-tick as shown below
- 4. Click Create Report

This opens a dialog to allow you to save the report, after which it will be opened in the form of a web page.

QIP Progenesis	QI.p Tutorial H	DMSe - Prog	genesis QI for pr	oteomics										
File														
Import Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Review Proteins	Protein Statistics	Report			nonlinear
	•			•			•	•			0			A Waters Company
Protein report	t Peptide repo	ort												
Report on	your protei	ns			Structure	the report								
	eport containir	ig the prote	eins of interest	in your	Enter a repor	t title:								
experiment.					Max Fold Inc	reased Proteir	ns							
	what to repo				Select the se	ctions to inclu	ude in the rep	ort:						
	ne list below, fil s you want to ir		ita to show only ie report.	/ the	Verv	iouv runo								
		@ Lean	n about tagging	and filtering										
O Curton	nise the repor	•			🔿 🔲 Data p	-								
📕 🖌 Enter a	title for your re	eport and se	elect the sectio	ns you want	List the proce	essing metho	ds used for pe	eak and peptide	ion detection					
to includ	de in it using th	ne controls	to the right.		🔿 🔳 Experi	ment design								
🔝 Tag f	ilter applied		_		<u> </u>	-	g the structur	e of the experim	ent i.e. the s	amples in each	experime	ental co	ndition	
T prote	ins may be hide	len		Edit	<u> </u>						_			
Accession	Anova (p)	Fold	Tag 🔻 D	escription	🔿 🔽 Protei									
Q9UVL1	6.06E-07	2.4		on-histone ch			otein abundar	nces and peptide	s identified fo	or each protein				
C4YL44	1.04E-06	2.2		tracellular ma	Prote									
P87219	2.18E-06	4.1		orbose reducta	Pept	de tables								
O9P8O7	4.09E-06	2.0		ocitrate lyase	🔿 🗷 Protei	n Details								
P42800	2.16E-05	2.7		ositol-3-phos	Reports the f	ull details of	every protein	which matches	your current	filter				
Q7Z8E8	2.39E-05	3.3	-	ell surface hyd	🔽 Tags									
C4YF25	3.44E-05	2.3	🍎 Bi	ud site selectic	🗷 Expre	ession profile								
C4YRH4	0.00108	2.1	-	AD(P)H-deper	👻 🔲 Peptic	le ion table								
C4YE92	0.0018	2.6	6	7-dimethyl-8-	Peptic									
Q5ALX8	0.00206	2.1	🍈 A	denine phospl	Peptic	le ion details								
Q59U83	0.00315	4.1	i Ri	bonucleoside	Create repor	t								
Q59NN8	0.00544	3.5	🌢 н	sp70 nucleotic										
Q9HFQ6	0.00581	2.2	60 🌔	S acidic ribos										
Q59WG0	0.0125	3.2	🥚 н	t family prote										
Q59Z58	0.188	10.6	Pr	otoplast secre										
		_												
4	m			+										
Experiment	design: A B C		•]										
	-			,										

Click on the **Accession No**. in the proteins section of the Report and this will take you to the Assigned peptides for this protein

Experiment: Progenesis QL.p Tutorial HDMSE Report creates: 02/09/2019 13:31:52 Protein Protein grouping Group similar proteins Protein guantitation Absolute Quantitation using H-3	Max Fol	d Increased Proteins						
Proteins Protein building options Protein grouping Group similar proteins Protein grouping Group similar proteins Protein quantitation Absolute Quantitation using Hi-3 Accession Peptides Score Anova Fold Tags Description Accession Peptides Score Anova Fold Tags Description A D D AD	Experiment:	Progenesis QI.p Tutorial HDMSe						
Protein building options Protein grouping <u>rouping inflar proteins</u> Containation Absolute Quantitation using H-3 Accession <u>peptides</u> <u>Score Anova</u> <u>fold Tags pescription</u> <u>Average Normalised</u> <u>A o total contained</u> <u>A co</u>	Report create	red: 02/09/2019 13:31:52						
Protein grouping Group similar proteins Protein quantitation Absolute Quantitation using HI-3 Accession Peptides Score Anova fold Tags Description Average Normalised Description Contracte lyase GN=ICL1 PE=3 SV=1 2.07 4.16 3.31	Proteins							
Protein grouping Group similar proteins Protein quantitation Absolute Quantitation using HI-3 Accession Peptides Score Anova fold Tags Description Average Normalised Description Contracte lyase GN=ICL1 PE=3 SV=1 2.07 4.16 3.31	Protein building	2 options						
Accession Peptides Score Anova (p)* Fold Tags Description Average Normalised Abundances 0292802 6 (d) 56.53 4.09e-006 2.01 isocitrate lyase GN=ICL1 PE=3 SV=1 2.07 4.16 3.31 02 OPPBQZ Isocitrate lyase GN=ICL1 PE=3 SV=1 2.07 4.16 3.31 02 Isocitrate lyase GN=ICL1 PE=3 SV=1 Isocitrate lyase GN=ICL1 PE=3 SV=1 Isocitrate lyase GN=ICL1 PE=3 SV=1 02 Sequence Peptide Score Hits Mass Charge Tags Conflicts Modifications In In Isocitrate lyase GN=ICL1 PE=3 SV=1 02 Accession QPB8Q7 Isocitrate lyase GN=ICL1 PE=3 SV=1 Peptides 6 (6) Score 56.53 Anova 4.09e-006 1 TDEAATI VIVPVQE Description Isocitrate lyase GN=ICL1 PE=3 SV=1 Peptides 6 (6) Score 56.53 Anova 4.09e-006 1 Gd 2.01 Image: Anova 2.05 Max fold change 2.2 Image: Anova 2.05 Max fold change 2.2	Protein groupir	ng Group similar proteins						
928202 6 (6) 56.53 4.09e-006 2.01 Isocitrate lyase GN=ICL1 PE=3 SV=1 2.07 4.16 3.31 92 Isocitrate lyase GN=ICL1 PE=3 SV=1 6 6 1	-	otides Score Anova Fold Tags Description Average Normalised						
QPP8Q7 Sequence Peptide ASADIFGSNLLAVAR 9787 7.39 9 Sequence Sequence VUPYQE Accession Q9P8Q7 Central TDSEAATL VUPYQEH Description Isocitrate lyase GN=ICL1 PE=3 SV=1 Peptides 6 (6) Score 56.53 Anova 4.09e-006 Fold 2.01 Anova 4.09e-006 Fold 2.01 Anova p-value ≤ 0.05 Max fold change ≥ 2 Max fold change ≥ 2 A								
P43 6 peptides P82 Sequence Peptide Score Hits Mass Charge Tags Conflicts Modifications In Drift Average Normalised Abundances Q21 ASADIFGSNLLAVAR 9787 7.39 9 1503.8215 2 0 yes 4.28 6636.02 1.81e-004 1.49e-004 C4 AYGQTVQ Accession Q9P8Q7 Constrained Description Isocitrate lyase GN=ICL1 PE=3 SV=1 Peptides 6 (6) Score 56.53 Anova 4.09e-006 Fold 2.01 Anova 4.09e-006 Fold 2.01 Anova p-value ≤ 0.05 Max fold change ≥ 2 A B C C C C C C C		27						
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Q2 ASADIFGSNLLAVAR 9787 7.39 9 1503.8215 2 0 yes 4.28 6636.02 1.81e+004 1.49e+004 AYGQTVQ LFHEAVID Q25 Accession Q9P8Q7 Accession Q9P8Q7 Accession Q9P8Q7 Image: Constraint of the second	Sequence	lon quantitation time						
LFHEAVID G4 TDSEAATL Q5 TDSEAATL VLVPVQEH VLVPVQEH WSGATYID Accession Q9P8Q7 Peptides 6 (6) Score 56.53 Anova 4.09e-006 Fold 2.01 ● Anova p-value ≤ 0.05 ● Max fold change ≥ 2	<u></u>	NLLAVAR 9787 7.39 9 1503.8215 2 - 0 yes 4.28 6636.02 1.81e+004 1.49e+004						
Description Isocitrate Iyase GN=ICL1 PE=3 SV=1 VI_VPVQEH VI_VPVQEH WSGATYID Anova 4.09e-006 Fold 2.01 Anova p-value ≤ 0.05 Max fold change ≥ 2 A B C	LFHEAVIDE	Association OOD207						
VLVPVQEF VLVPVQEF WSGATYID Anova 4.09e-006 Fold 2.01 ● Anova p-value ≤ 0.05 ● Max fold change ≥ 2	TUSEAATL	1						
Anova 4.09e-006 Fold 2.01 Anova p-value ≤ 0.05 Max fold change ≥ 2 A B C		Peptides 6 (6)						
Anova p-value ≤ 0.05 Max fold change ≥ 2 A B C		Anova 4.09e-006						
	Max fold change ≥ 2							
3.0 2.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1								
		3.0 T H H H H H H H H H H H H H						
VICTOR VI		HILD THE ACCENT OF A CONTRACT OF						

Having closed the report it can be reopened by double clicking on the saved html file.

Note: you can also copy and paste all or selected sections of the report to Excel and/or Word.

Note: there are separate panels for reporting on Proteins and Peptides.

Appendix 1: Stage 1 Data Import and QC review of LC-MS data set

You can use your own data files, either by directly loading the raw files (Waters, Thermo, Bruker, SCIEX and Agilent) or, for other Vendors, convert them to mzXML or mzML format first.

To create a new experiment with your (Waters) files: open Progenesis QI for proteomics and click **New**, bottom left of the **Experiments** page and give your experiment a name. Then select data type, the default is 'Profile data'.

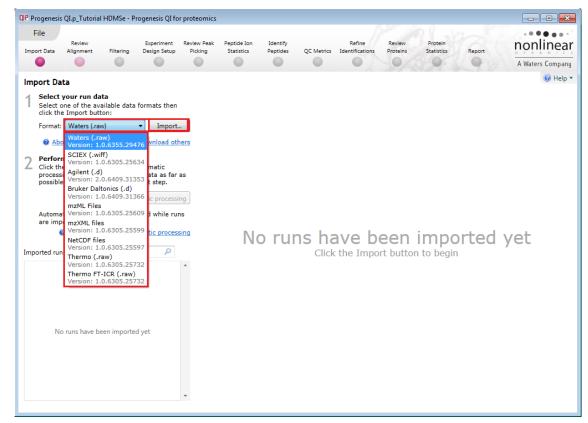
Note: if you have converted or captured the data as centroided then select Centroided data and enter the Resolution for the MS machine used.

OP Create New LC-MS Experiment		
Create a new label-free experiment named:		
Progenesis QI.p Tutorial HDMSe		
_Data type		
Profile data		
Centroided data		
Resolution (full width at half maximum) 50000]	
Machine type		
High resolution mass spectrometer	•	
Experiment folder		tion mass spectrometer
Save experiment in the same folder as the run data	- Í	APT G2/G2-S, AB SCIEX TripleTOF, Agilent QTOF, Bruker Maxis, Thermo LTQ Orbitrap
Choose an experiment folder	Thermo LTC	l Iontrap in Enhanced mode.
	Low resolut	ion ion trap
		Bruker HCT Ultra, Thermo LTQ XL
Create experiment	Thermo FT-	ICR

Click **Create experiment** to open the LC-MS Data Import stage of the workflow.

Select the 'Import Data file format', in this example they are Waters/SYNAPT data

Then locate your data files using Import...



	Import Waters .RAW Data
	Select your runs The data for each run is stored in a separate .RAW folder, usually all within the same containing folder.
Locate and select all the .RAW folders (A_01 to C_03).	Find runs in folder: D:VProgenesis v3.0 HDMSe Tutorial/QIp_Tutorial HDMSe v3.0 Browse A_01 rew A_02 rew B_01 rew B_01 rew B_02 rew B_03 rew C_01 rew C_02 rew C_03 rew
	< Back Next > Cancel
	Import Waters .RAW Data Lock mass calibration
	All of your runs contain lock mass calibration information. Please provide the calibration m/z.
	Perform lock mass calibration
	Note: If you have already calibrated your data externally, you should not perform calibration here.
On importing, the lock mass calibration is read and presented on this dialog	
You can, if required, alter the lock mass calibration at this step.	
	< <u>B</u> ack <u>Next</u> > Cancel

For MSe, HDMSe and SONAR data formats the Ion accounting workflow is selected as default if your computer has a GPU.

Click Next. You can either choose to calculate optimal thresholds using an appropriate FASTA file for your data set or set the Thresholds manually.

Import Waters .RAW Data	Import Waters .RAW Data
Enable Ion Accounting workflow To identity peptides in your MS≢/HDMS≢/SONAR data, you will need to enable the Ion Accounting workflow.	Optimise peptide identification Specify the peak intensity thresholds to use when filtering your spectra prior to searching with Ion Accounting.
Select whether to run the Ion Accounting workflow.	How do you want to specify the threshold intensities?
Run the Ion Accounting workflow and associated data analysis	 Calculate optimal thresholds using a representative FASTA file
	FASTA file: E:\HDMSe Tutoria\\Tutorial_nd_DB.fasta Browse
	Parsing rules: UNIPROT
	Specify threshold intensities manually
	Low energy: 250 counts
	Elevated energy. 150 counts
< Back Next > Cancel	How does the automatic calculation of thresholds work? (<u>B</u> ack <u>N</u> ext> Cancel

If you choose to determine the thresholds automatically then Progenesis determines appropriate thresholds by sampling each run and finding the thresholds that yield the most protein identifications in the sample area. For each run, Progenesis performs the following steps:

- It finds the 5-minute retention time window that contains the highest total intensity. •
- It extracts the ions within this window and performs multiple Ion Accounting searches, each one using a different set of threshold values.

 Finally, it selects the thresholds that resulted in the largest number of protein identifications and applies those to the whole run. If more than one set of thresholds results in similar numbers of identifications (within 10% of the maximum), it will choose the highest thresholds as a way of optimising system performance.

Note: for HDMSe the default settings are 150 and 30 and for MSe the default settings are 250 and 150 for the Low and Elevated energies respectively.

Having selected how to handle the thresholds you will get the option to specify your own elution limits, the default is Start and End of the run, accept or make changes as necessary.

Import	: Waters .RAW Dat	a			
		Ion Accounting workf i the defaults are not appro		orkflow.	
	you choose to spe le start time.	cify your own elution limits	, the end time m	ust be at least 5 n	ninutes after
	Elution start:	<start of="" run=""></start>	minutes		
	Elution end:	<end of="" run=""></end>	minutes		
			< <u>B</u> ack	<u>N</u> ext >	Cancel

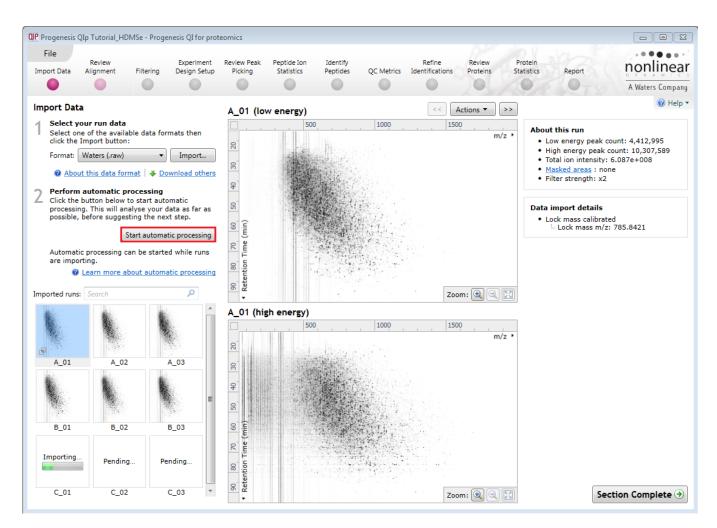
Ready to				
Please re	view the information below before	ore starting the import process		
Your runs	are ready to be imported. Plea	se review the options below.		
1 run s	elected for import.			
	ass calibration: Yes t mass m/z: 785.8426			
Thre FAS	sing parameters: shold mode: Automatic TA file: E:\HDMSe Tutorial\Tut TA parsing rules: UNIPROT	torial_nd_DB.fasta		
		< Back	Import	Cancel

A summary of the loading parameters is provided before you click Import

On loading the selected runs your data set will be automatically examined and the size of each file will be reduced by a 'data modelling routine', which reduces the data by several orders of magnitude but still retains all the relevant quantitation and positional information.

Note: For a large number of files this may take some time.

Note: you can start the automatic processing before the loading has completed.



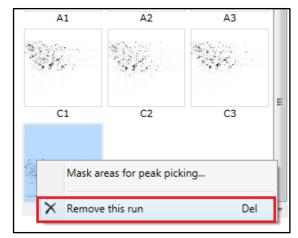
For details of setting up the steps in the automatic processing wizard return to Stage 2A page 7.

Review Chromatography

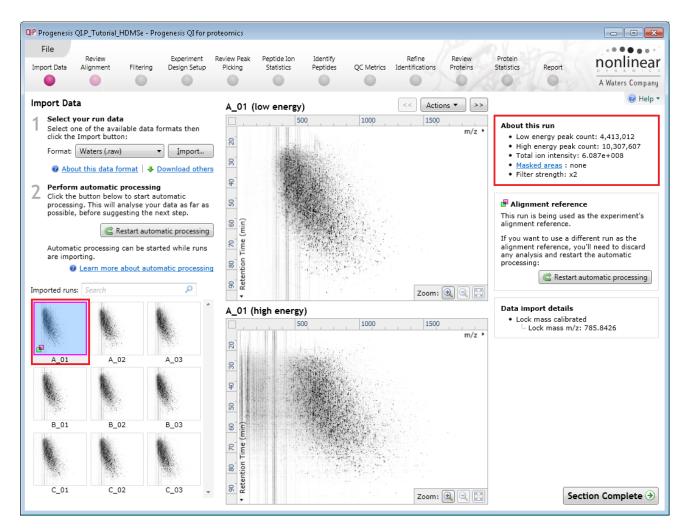
Each data file appears as a 2D representation of the run. If you created a **profile** experiment, at this stage you will be warned if any of the data files have been 'centroided' during the data acquisition and conversion process, as files must be of one format or the other.

You can delete run(s) by left clicking on the run in the list.

Note: you can also multi-select runs to remove by holding down the Ctrl key.



At the Import Data stage you can examine the quality of the imported runs using the 2D representation of the runs



Note: details of the current run appear on the top right of the view.

Once you have reviewed the imported runs click on **Review Alignment** on the workflow or **Section Complete** to move forward to the Review Alignment Stage.

Note: you will be offered the automatic alignment if you have not performed it automatically already.

Now move to the next stage in the workflow (page 7 in this user guide).

Appendix 2: Stage 1 Processing failures

During automatic processing if a stage fails to complete successfully or only partially completes, the automatic processing dialog will warn you of the problem. Depending on the type of failure this may or may not allow the automatic processing to complete.

For example, a run that fails to automatically align will trigger a warning, although analysis will continue; however, the automatic processing dialog will prompt you to 'drop-off' at the **Review Alignment** stage on completion to investigate the problem.

OP Processing Complete		X
Automatic process Time taken: 3 minutes 9	ing complete (with warnings). seconds	
 Importing runs: Selecting reference: 	7 of 7 processed C1	
Aligning runs:	6 of 6 processed A 1 run failed to align - continuing without it	
Peak picking:	14624 peaks found	
 Creating design: 	Created	
 Protein quantitation: 	Relative Quantitation using Hi-3	
	Close Identify Peptide	es 🌖

Note: in this example the run that failed to align will not contribute to the peak picking and will be excluded at the alignment stage (a cross appears in the include column).



You can either remove the run from the experiment at the **Import Data** or add it back in at the **Review Alignment** stage once the alignment of the run has been corrected.

As another example, runs that import successfully but with warnings at the **Import Data** stage will cause a flag in the readout to notify you of the potential quality issue.

If some runs in a data set fail to import (but not all), the automatic processing will continue informing you that one or more runs have failed to import.

QIP Processing Complete		×
Automatic process Time taken: 3 minutes 40	ing complete (with seconds	warnings).
A Importing runs:	7 of 7 processed A 1 failed to import	
 Selecting reference: 	C1	
 Aligning runs: 	5 of 5 processed	
 Peak picking: 	14624 peaks found	
 Creating design: 	Created	
 Protein quantitation: 	Relative Quantitation us	ing Hi-3
		Close Identify Peptides 🕥

In this case you can remove the runs at Import Data and if appropriate replace them with additional runs.

Note: adding additional runs will then be aligned and peak picking should be re-done to include data from the added runs in the generation of the aggregate.

An example of a problem that would halt the automatic processing would be the failure to successfully import all the potential reference candidates, (for example: while importing, you specified the selection of the alignment reference to be made from several runs before they were fully imported and set the processing underway, and they later failed to import owing to problems with the runs).

QIP Proces	sing Complete		×
	atic process en: 19 seconds	ing failed.	
🛦 Impor	ting runs:	6 of 6 processed A 1 failed to import	
× Select	ing reference:	All reference candidates failed to import	
Aligni	ng runs:	Unable to start.	
Peak	picking:	Unable to start.	
Protei	in quantitation:	Unable to start.	
		Close	Import Data 🌖

In this case, the processing dialog would halt and prompt you to select another reference.

Appendix 3: Licensing runs (Stage 3)

When setting up a **New experiment** if you are evaluating Progenesis QI for proteomics with unlicensed runs then the licensing page will open after **Import Data section**.



If you already have a programmed dongle attached to your machine then the License Runs page will not appear.

To use this page to License your Runs you must first either obtain an 'Evaluation' Licence Code from a Sales Person or purchase a licence code directly.

Each code will allow you to license a set number of runs.

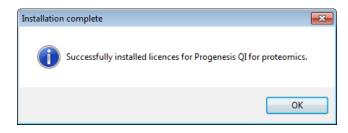
The runs in your experiment will be listed as shown below.

To activate license(s) for the selected runs enter the code in the space provided and click **Use Licence code**.

Note: you will need an internet connection to use this method.

File Review Experiment mport Data Licensing Alignment Filtering Design Set	up Picking Statistics Peptides QC Metrics Identifications Proteins Statistics Report	Waters Comp
Dongle License Runs		
This installation is currently restricted to analyse licensed runs only.	Run name Licence state	License this run
To license your runs, you need an evaluation or	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	V
lease licence code which can be obtained from	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis Q1.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	V
a sales representative.	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	
Once licensed, your runs can be analysed on	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis Q1.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	V
any installation of the software. The licence is	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	
automatically included when archiving an	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	V
experiment.	C:\Users\andy.borthwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	
If your runs have been licensed on another computer, click here to make the licences	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	
available on this computer.	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis Q1.p v3.0 Tutorials and Demo Suites\Pr Unlicensed	V
If you have one, you can <u>open a licence file</u> to install. If you have just installed a dongle, <u>click here</u> .		
	Run licence code: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx	ice Code

A message confirming successful installation of your licences will appear.

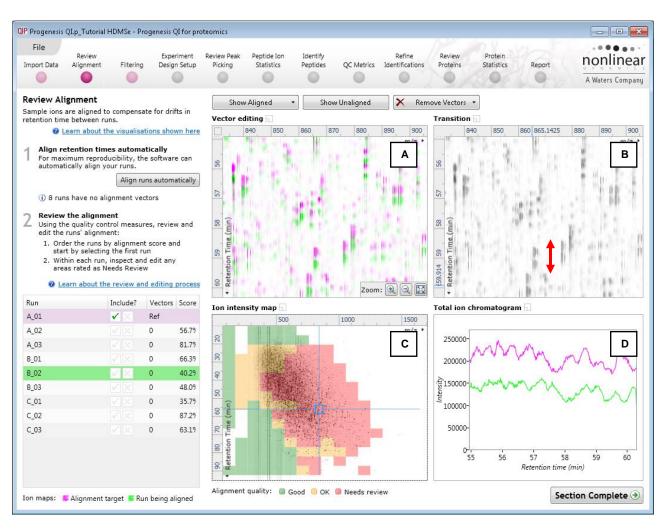


Click OK, the view will update and Alignment, the next stage in the workflow, will open with the licensed files.

Appendix 4: Manual assistance of Alignment

Approach to alignment

To place manual alignment vectors on a run (B_02 in this example):



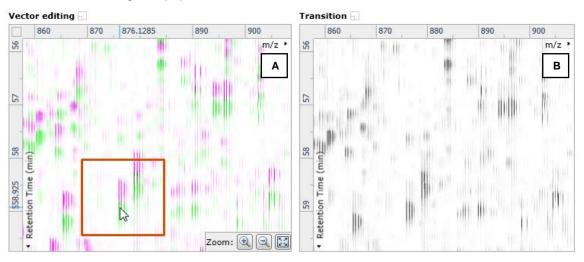
- 1. Click on Run B_02 in the **Runs** panel, this will be highlighted in green and the reference run (A_01) will be highlighted in magenta.
- 2. You will need to place approximately 5 10 **alignment vectors** evenly distributed from top to bottom of the whole run (RT range).
- 3. First drag out an area on the **Ion Intensity Map** (C), this will reset the other 3 windows to display the same 'zoomed' area

Note: the peptide ions moving back and forwards between the 2 runs in the **Transition** window (B) indicates the misalignment of the two runs.

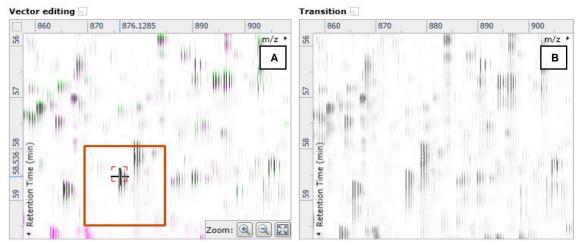
Note: the lon Intensity Map gives you a colour metric, visually scoring the current alignment and an overall score is placed next to the Vectors column in the table. With each additional vector added this score will update to reflect the 'changing' overall quality of the alignment. The colour coding on the lon intensity Map will also update with each additional vector.

Note: The **Total Ion Chromatograms** window (D) also reflects the misalignment of the 2 runs for the current Retention Time range (vertical dimension of the current Focus grid in the **Ion Intensity Map** window).

4. Click and hold on a green peptide ion in Window A as shown below.

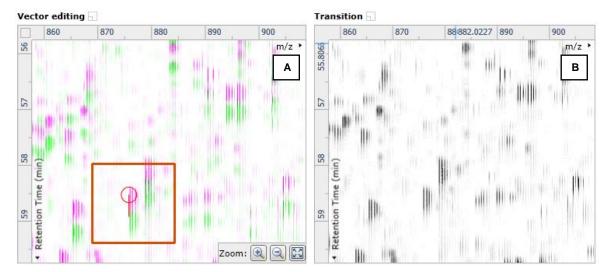


5. As you are holding down the left mouse button (depending on the severity of the misalignment), the alignment vector will automatically find the correct lock. If not, drag the green peptide ion over the corresponding magenta peptide ion of the reference run. The red box will appear as shown below indicating that a positional lock has been found for the overlapping peptide ions.



Tip: while holding down the mouse button hold down the **Alt** key. This will allow smooth movement of the cursor as the **Alt** key allows you to override the 'automatic alignment' performed as you depress the mouse button.

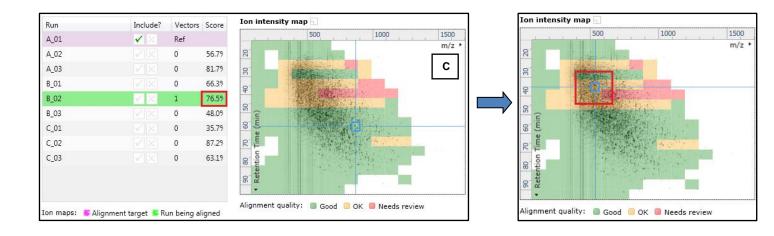
6. On releasing the left mouse button the view will 'bounce' back and a red vector, starting in the green peptide ion and finishing in the magenta peptide ion will appear.



Note: an incorrectly placed vector is removed by right clicking on it in the **Vector Editing** window and selecting delete vector.

- Transition Vector editing 860 875.9839 900 880 900 870 890 860 870 890 56 m/z • 56 m/z • в Α 22 27 59 58.536 58 58 Retention Time (min) (mim) Retention Time 59 Zoom: ۲
- 7. Now click **Show Aligned** on the top tool bar to see the effect of adding a single vector.

8. With the placement of a single manual vector the increase in the proportion of the **Ion Intensity Map** (C) showing green is reflected in the improved alignment score in the table. Now click in the Ion Intensity Map to relocate the focus in order to place the next manual vector.



9. Adding an additional vector will improve the alignment further as shown below.



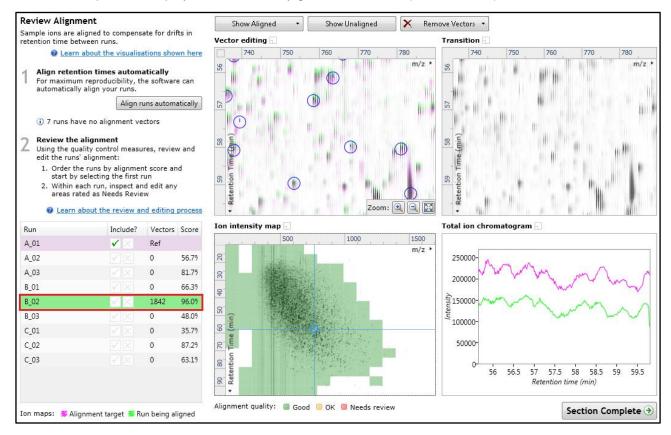
- 10. The shift in the Retention Time (RT) is as a result of incorrect running of the chromatography. In many of these cases if the Automatic Alignment fails to generate a good alignment then removing all the alignment vectors for this run and placing a single manual vector to act as a 'seed' for the Automatic Alignment algorithm maybe all that is required to generate a good alignment.
- 11. In the case of the example shown above placing a small number of vectors from the top to the bottom of the run is sufficient to markedly improve the alignment.



 At this point you would redo the automatic alignment of this image by selecting automatic alignment. Note: if you are focusing only on the alignment of one run, then un-tick the other runs in the alignment dialog.

Automatic Al	ignment	—
Choose whic	ch runs to automatically align:	
Run	Notes	Vectors
A_02	This run has not been automatically aligned	0
🗖 A_03	This run has not been automatically aligned	0
🔲 B_01	This run has not been automatically aligned	0
✓ B_02	run has user vectors	5
🗖 B_03	This run has not been automatically aligned	0
🗖 C_01	This run has not been automatically aligned	0
🗖 C_02	This run has not been automatically aligned	0
🔲 C_03	This run has not been automatically aligned	0
		OK Cancel

13. On pressing OK the Automatic Alignment will run for the selected run. On completion the table and views will update to display the automatically generated vectors (shown in blue).



14. Repeat this process for all the runs to be aligned.

The number of manual vectors that you add at this stage is dependent on the misalignment between the current run and the Reference run.

Note: In many cases only using the Automatic vector wizard will achieve the alignment.

Tip: a normal alignment strategy would be: to run the automatic alignment first for all runs, then order the alignments based on score. For low scoring alignments remove all the vectors and place 1 to 5 manual vectors to increase the score then perform automatic alignment. Then review the improved alignment score.

Also the 'ease' of addition of vectors is dependent on the actual differences between the LC-MS runs being aligned.

To review the vectors, automatic and manual, return to page 18

Appendix 5: Within-subject Design

To create a **Within-subject Design** for your data set select this option on the **Experiment Design Setup** page and enter the name of the design.

In this example there are 3 Subjects (i.e. patients A, B and C) who have been individually sampled: Before(1), During (2) and After (3) treatment

File nport Data	Review Alignment	Filtering	Experiment Design Setup	Review Peak Picking	Peptide Ion Statistics	Identify Peptides	QC Metrics	Refine Identifications	Review Proteins	Protein Statistie		port	D	onlin Waters Cor
B C Which ex	A B	Ne lesien tvo		ant to use	for this ex	periment?								
00 00 Do samp appear in	etween-su les from a gi n only one co between-sub	ubject De	esign hen A			Delete	0-0 Have y subjec	Within-sub ou taken samp t under differe ise the within-	ples from a g ent condition	iven 15?		Before	During	After
To set up the runs (factor le	this design, according to evel) of the sa alculation ass	you simply the condition amples. Th	group on e	Ente	eate New Experi r a name for th	ne experiment d	lesign:		×	use	Patient X	X1	X2	X3
therefore	ns are indepe e gives a stat the means of qual.	istical test o		How		After Treatment o group the run s manually			~	s	Patient Y	Y1	Y2	Y3
			Add	conditi	Copy an existir	ng design:	C	reate design	Cancel	ut he ted	Patient Z	Z1	Z2	Z3
							becaus assump repeat differe reduce conditi create The wi though paired- compa	dard ANOVA is a e the data viol tion of indepe d measures Al nces can be eli d as a source o on differences a more powerf thin-subject de t of as an exte samples t-test rison between d measures.	ates the ANO ndence. With NOVA individu iminated or f between (which helps ful test). esign can be nsion of the to include	te VA a Jal				

When the design page opens use the **Add Subject** and **Add Condition** buttons to create the matrix that fits your experimental design, over typing the names as required.

Then Drag and drop the Samples on to the correct 'cell' of the matrix.

	eomics iew Peak Peptide Id Vicking Statistici		Refin QC Metrics Identifica		Protein Statistics Report	nonlinear
						A Waters Company
A B C A B Before During an	nd After Treatm	nent I × 📄	New			🕢 Help 🔻
Setup conditions and subjects Setup the conditions and subjects for your experiment design on the right, and then assign each of your samples to the correct subject/condition cell in the grid.		Before	During	After	Add Condition	
 Add a column for each condition. Add a row for each subject. Drag each of your samples to the correct location in the grid. Filter samples: P	Patient A	A_01	A_02	A_03		
C_02	Patient B	B_01	B_02	B_03		
C_03	Patient C	C_01	Select Sample	Select Sample		
	Add Subject				-	
					S	ection Complete 🏵

You can create additional Experimental Designs using the New tab

All of these Experimental Designs are available at the later stages of the workflow with the exception of **Identify Peptides**, and **Refine Identifications** (including Resolve Conflicts).

Appendix 6: Power Analysis (Peptide Ion Stats)

Power analysis is a statistical technique that is used to gauge how many replicates are needed to reliably see expression differences in your data. It is available through the Peptide Ion Stats section of the workflow.

To perform a power analysis of the data click on **Ask another question** at the top of the table in the Peptide Ion Stats screen. A selection of 3 tools will appear in the form of questions.

v	Principal Components Analysis Are there any outliers in my data? Does my data cluster according to my experimental conditions?
ጨ	Correlation Analysis Group my quantifiable proteins according to how similar their expression profiles are.
	Power Analysis How many replicates should I run? What is the power of my experiment?

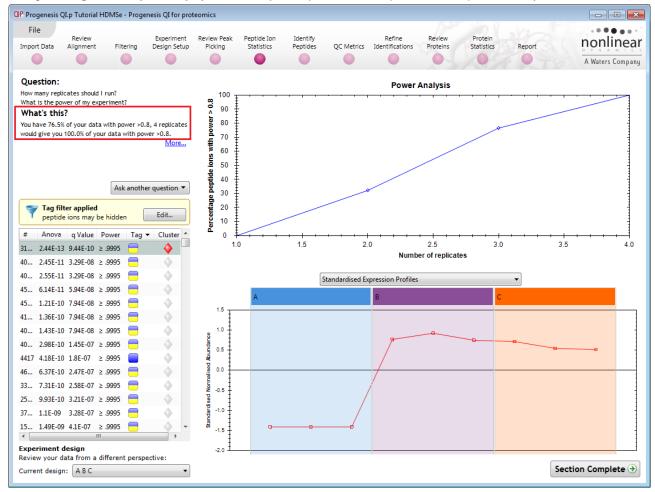
Select the option

'How many replicates should I run and what is the power of my experiment?'

It answers this question by informing you:

'How many replicates you need so that at least 80% of your peptide ions with a power >0.8'

Using the Significant p<0.05 peptide ions (20641), as an example, view the power analysis.



This is displayed graphically showing that 76.5% of the 20641 peptide ions have a power of 80% or that 4 replicates would give you 100% of your data with power > 0.8.

- The power of a statistical test reflects our confidence in the experimental data's ability to find the differences that do actually exist
- The power is expressed as a percentage, where 80% power is an accepted level, therefore allowing you to assess the number of sample replicates that would be required to achieve a power of 80%.

Appendix 7: Resolve Conflicts

This stage allows you to examine the behaviour of the identified peptides and choose to resolve any conflicts for the various peptide assignments at the protein level.

The Resolve Conflicts stage is now accessed at the bottom left of the Refine Identifications stage.

The number of conflicts you have to resolve will depend on the scope and stringency of the filters you apply at the **Refine Identifications** stage.

Note: the default Protein options for protein grouping and Protein quantitation are set as shown

QIP Protein quantitation options	×
Quantitation method:	
Relative Quantitation using Hi-N	•
Number of peptides to measure per protein (N):	3 🔹
Employ protein grouping, i.e. hide proteins whose peptides are a subset of another protein's.	
OK Canc	el

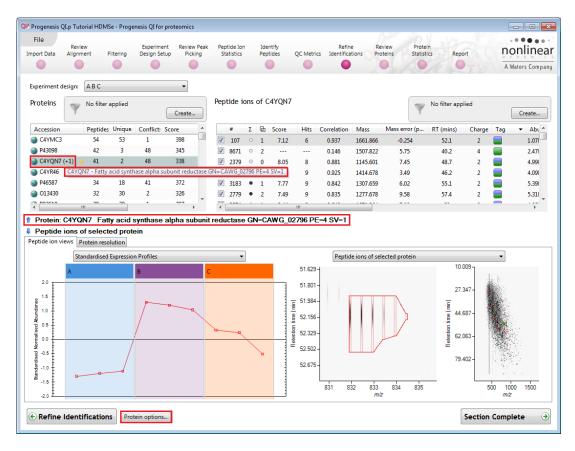
This means that if you choose **not** to resolve the conflicts then proteins, to be considered for quantitation, require at least one unique peptide (see Unique column).

For this guide: we will not resolve the conflicts but click on Section Complete to go to Review Proteins (page 61).

(For more details on Protein Grouping page 56 and Protein Quantitation options go to page 58).

If you wish/require to resolve conflicts then the remainder of this appendix provides some guidance and explanation of performing this process in Progenesis QI for proteomics.

With **Group similar proteins** selected the additional members are indicated by a bracketed number located after the Accession number.

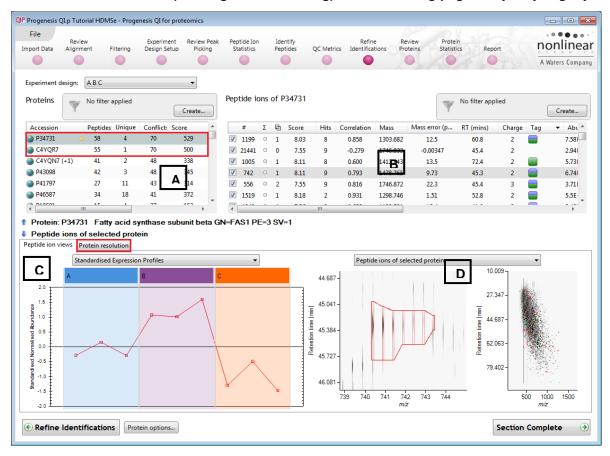


The number of the **Peptides** used for quantitation is displayed in the **Unique** column.

The **Resolve Conflicts** stage provides a number of interrelated graphical and tabular views to assist you in the validation of the peptides that have been assigned to proteins and also to review the relevance of the data returned from the search.

With Resolve Conflicts open order the data in the Proteins table A on the basis of Conflicts.

Note: the look of the tables (with regards to ordering) in the following pages may vary slightly.



Select the first protein in list A (in this case it has 70 conflicts) the panel to the right B lists the peptides for this protein and the conflicting protein for each peptide.

	Accession	Peptid	es Unique	Conflict	Score	•		#	Σ	몓	Score	Hits	Correlatio	n Mass	Mass error (p	RT (mins)	Charg	e Tag	✓ Abi ^
	🍛 P34731	<u> </u>	4	70	529	-	V	1199	0	1	8.03	8	0.858	1303.68	2 12.5	60.8	2		7.58
	C4YQR7	55	1	70	500		V	21441	0	0	7.55	9	-0.279	1746.83	3 -0.00347	45.4	2		2.941
	C4YQN7 (+1)	41	2	48	388		V	1005	0	1	8.11	8	0.600	1412.84		72.4	2		5.731
	P43098	42	3	48	A 315			742	0	1	8.11	9	0.793	1478.76	5 9.71 E	45.3	2		6.741
	P41797	27	11	43	514	-	V	556	0	2	7.55	9	0.816	1746.87	2 22.3	45.4	3		3.711 👻
	•					•	•	_	_	_	_	III		_					F.
ſ	Protein: P34	731 Fat	ty acid s	ynthase	subunit be	eta Gl	N=FA	AS1 P	E=3	SV	=1								
Ļ	Protein: C4Y	QR7 Fa	atty acid	synthase	e beta sub	ounit c	lehy	dratas	se G	iN=(CAWG_	04414	PE=4 SV	=1					
Ρ	eptide ion views	Protein re	solution																
	Conflicting p	roteins f	or peptio	le ion 742	² E	F	eptio	de ion	s of	C4	YQR7					F			
	Accession	Peptides	Unique	Conflict: P	rotein Score	I	#	Σ	ę	s So	core	Hits	Correlation	Mass	Mass error (p	RT (mins)	Charge	Tag	✓ Abun ^
	🕲 P34731 🛛 🍳	58	4	70 52	9	[7	42 •	1		8.11	9	0.779	1478.765	9.73	45.3	2		6.74E+
	C4YQR7	55	1	70 50	0	[√ 5	56 0	2		7.55	9	0.813	1746.872	22.3	45.4	3		3.71E+
						[v 10	005 0	1		8.11	8	0.633	1412.843	13.5	72.4	2		5.73E+
						[✓ 62	204 0	1		7.52	8	0.851	1380.691	9.88	27	3		1.69E+
							✓ 1 <u>5</u>	5 19 🖸	1		8.18	2	0.940	1298.746	1.51	52.8	2		5.5E+(👻
	•					•	•		-	-									•

Panel C shows the expression profile(s) for the peptide(s) selected in list B

Panel D shows the details for the selected peptide.

Now click on the Protein Resolution in Panel C to display the proteins that are conflicting.

The lower left panel E displays the Conflicting proteins for the peptide ion highlighted in panel B this includes the current protein in panel A as indicated by the orange ball to the right of the accession.

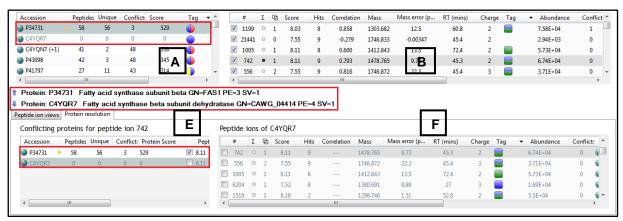
The Accession and description for the 2 proteins highlighted in Panels A and E are shown in the middle

margin. As most of the peptide ions are conflicting between the 2 closely related proteins one simple way to resolve these conflicts is to favour the protein with the higher score and greater number of non-conflicting peptides.

One way to do this is to right click on the lower scoring protein in panel E which only has one unique peptide and turn off all its peptides

Accession		Peptides	Unique	Conflict:	Prote	in Score
🎯 P34731	0	58	4	70	529	
🕥 C4YQR7		55	1	70	500	
		Т	urn off al	l peptides		1
•	Ш					•

All the peptides are now switched off in panel B and all the entries for the lower scoring protein are set to zero. The higher scoring protein now has 56 non-conflicting peptides and only 4 remaining conflicts



To resolve the remaining conflict(s) first order the conflicts in panel B and select the top one (which may still be selected) Panel B will display the peptides for this protein and the number of conflicts for each peptide. Panel E will also update to show the conflicting protein.

Accession	Peptid	es Uniqu	e Conflic	t: Score	Tag	* ^		#	Σ	中	Score	Hits (Correlation	Mass	Mass error (p.	 RT (mins) 	Charg	je Tag	 Abundance 	e Co	onflict
P34731	o 58	56	3	529			V	2183		0	5.9	2	0.718	1529.861	3.14	45	3		5.05E+04		1
C4YQR7	0	0	0	0			V	1253	0	2	5.9	2	-0.176	1529.827	-19.2	44.9	2		7.22E+04		1
C4YQN7 (+1)	41	2	48	338			V	1199	0	1	8.03	8	0.858	1303.682	12.5	60.8	2		7.58E+04		1
P43098	42	3	48	345			1	21441	0	0	7.55	9	-0.279	1746.833	-0.00347	45.4	2		2.94E+03		0
P41797	27	11	43	314		-	V	1005	0	1	8.11	8	0.600	1412.843	13.5	72.4	2		5.73E+04		0
						•															
Protein: P347 Protein: C4Y(otide ion views	QP9 60 Protein re	S ribos	omal pro	otein L35 GN		G_02	808 P	E=4 \$	S V =1		/000										
Protein: C4Y(otide ion views Conflicting pro	QP9 60 Protein re oteins f	IS ribos solution or pepti	omal pro	otein L35 GN	I=CAW(G_02		E =4 \$ de ior	SV=1	f C41											
Protein: C4YC tide ion views Conflicting pro	QP9 60 Protein re oteins f	IS ribos solution or pepti	omal pro	otein L35 GN		G_02	808 P	E =4 \$ de ior	SV=1	f C41	(QP9 ore Hit	s Corr	relation M	1ass N	Aass error (p	RT (mins)	Charge	Tag	✓ Abundance	Confli	ict:
Protein: C4Y(tide ion views Conflicting pro Accession	QP9 60 Protein re oteins f	IS ribos solution or pepti	de ion 2 Conflict:	otein L35 GN	I=CAW(G_02	808 P Peptic	E=4 \$ de ior Σ	SV=1	f C41				1ass N 314.714	Aass error (p 8.24	RT (mins) 49.1	Charge 2	Tag	 Abundance 1.3E+05 	Confli 1	_
Protein: C4YC itide ion views Conflicting pro Accession P34731 •	QP9 60 Protein re oteins f Peptides	OS ribos solution or pepti Unique	de ion 2 Conflict:	otein L35 GN 183 Protein Score	I=CAW(Pe	G_02	808 P Peptic	E=4 \$ de ior Σ	sv=1	f C41	ore Hit	0	0.702 13				Charge 2 3			Confli 1 1	(
Protein: C4Y(titide ion views Conflicting pro Accession P34731 C4YQP9	QP9 60 Protein re oteins fr Peptides 58	DS ribos esolution or pepti Unique 56	de ion 2 Conflict:	ntein L35 GN 183 Protein Score 29 55.2	I-CAW(Pe ▼ 5.9	G_02	808 P Peptic # 1 21	E=4 \$	SV=1	f C41	ore Hit 8.32 2	- 0).702 13).934 15	314.714	8.24	49.1	2		1.3E+05	1	(
Protein: C4Y(otide ion views Conflicting pro Accession	QP9 60 Protein re oteins fr Peptides 58 3	DS ribos esolution or pepti Unique 56 1	de ion 2 Conflict:	ntein L35 GN 183 Protein Score 29 55.2	I−CAW (Pε ▼ 5.9 ▼	G_02	808 P Peptio # ♥ 1 ♥ 21 ♥ 12	E=4 \$ de ior Σ 54 0 83 0 253 0	SV=1	f C4)	ore Hit 8.32 2	0 - 0 0	0.702 13 0.934 15 0.750 15	314.714 529.861	8.24 20.5	49.1 45	2		1.3E+05 5.05E+04	1	(

Favouring the protein with the higher score, but this time resolve the conflict by switching off (or unassigning) the peptide in panel F for the protein with the lower score. By doing this the other 3 panels update to show the change in conflicts.

Accession	Peptid	es Uniqu	e Confli	ct: Score	Tag	•		#	Σ	Φ	Score	Hits	Correlation	Mass	Mass error (p	RT (mins)) Char	ge Tag	 Abundance 	Cor	nflict ^
P34731	o 58	57	2	529		-		2183		0	5.9	2	0.718	1529.86	1 3.14	45	3		5.05E+04		0
S C4YQR7	0	0	0	0			V	1253	0	2	5.9	2	-0.176	1529.82	7 -19.2	44.9	2		7.22E+04	1	1
C4YQN7 (+1)	41	2	48	338			V	1199	0	1	8.03	8	0.858	1303.68	2 12.5	60.8	2		7.58E+04	1	1
P43098	42	3	48	345			\checkmark	21441	0	0	7.55	9	-0.279	1746.83	3 -0.00347	45.4	2		2.94E+03	(0
P41797	27	11	43	314		-	V	1005	0	1	8.11	8	0.600	1412.84	3 13.5	72.4	2		5.73E+04	(- 0
•	111				+	•															•
Peptide ion views Conflicting pr			de ion 2	183		I	Peptic	le ion	ns of	C4Y(QP9										
Accession	Peptides	Unique	Conflict:	Protein Score	Pep	ot	#	Σ	Ð	Sco	re H	its C	Correlation I	Mass	Mass error (p	RT (mins)	Charge	Tag	 Abundance 	Conflic	ct: Pep
🔇 P34731 🛛 🍳	58	57	2	529	☑ 5.9		1	54 🖸	1	8	.32 2	2	0.594 1	1314.714	8.24	49.1	2		1.3E+05	1	3
C4YQP9	3	1	2	25.2] [21	.83 0	0	-			1	1529.861	20.5	45	3		5.05E+04	0	3
S C4YQR7	0	0	0	0	5.9		12	53 0	2	8	.59 2	2	0.830 1	1529.827	-1.89	44.9	2		7.22E+04	1	0
							30	56 •	3	8	.34 2	2		168.709	13.4	49.9	2		1.1E+05	0	١
•					+		•					_									

Repeat this process until there are no conflicts remaining for the current protein in Panel A.

Now repeat using a similar approach for the next protein in Panel A, here the situation is similar.

Accession	Peptides	Unique	Conflict	Score	Tag 🔹	-	#		Σ	日 Scor	e Hi	its Correlatio	n Mass	Mass error (p.	RT (mins)	Charg	e Tag	g 💌 Abundance	e Cor	nflict_
P34731	58	58	0	529			V 1	.07		1 7.	12 6	5 0.932	1661.866	-0.254	52.1	2		1.07E+05	2	2
C4YQR7	0	0	0	0			V 8	671	0	2		- 0.093	1507.822	5.75	40.2	4		2.47E+04	2	2
C4YQN7 (+1)	41	2	48	338	•		2	379	0	0 8.0)5 8	3 0.910	1145.601	7.45	48.7	2		4.99E+04	1	1
P43098 o	42	3	48	345			2	779	•	2 7.4	19 9	0.866	1277.678	9.58	57.4	2		5.31E+04	1	1
P41797	27	11	43	314		-	2	930	0	0 8.1	13 9	0.937	1414.678	3.49	46.2	2		4.09E+04	1	L ·
(III				+		•													•
Protein: P4309 Protein: C4YQI ptide ion views	N7 Fat	ty acid								02796 F	PE=4 SN	V=1								
Protein: C4YQ	N7 Fat	ty acid	synthas	e alpha sub		uctas	e GN=	CAW	/G_	02796 F		V=1								
Protein: C4YQI ptide ion views Pr Conflicting prot	N7 Fat rotein reso teins for	ty acid	synthas e ion 10	e alpha sub		uctas P	e GN=	CAW ions	/G_				Mass 1	Mass error (p	RT (mins)	Charge	Tag	✓ Abundance	Conflict	te 🖌
Protein: C4YQI ptide ion views Pr Conflicting prot	N7 Fat rotein reso teins for	ty acid	e ion 10 Conflict:	e alpha sub 7	unit red	P	e GN=	CAW ions Σ	G_	C4YQN7			Mass / 1661.866	Mass error (p -0.254	RT (mins) 52.1	Charge 2	Tag	 Abundance 1.07E+05 	Conflict 2	t:
Protein: C4YQI ptide ion views Protection Pr	N7 Fat rotein reso teins for Peptides	ty acid	e ion 10 Conflict:	e alpha sub 7 Protein Score	unit red Pe	P I	e GN= eptide #	ions Σ	/G_ of (₪ 1	C4YQN7 Score	Hits	Correlation					Tag			t:
Protein: C4YQI ptide ion views Pr Conflicting prot Accession	N7 Fat rotein reso teins for Peptides 42	ty acid olution r peptid Unique 3 2	e ion 10 Conflict:	e alpha sub 7 Protein Score 345	Pe	P P 2 2	eptide # 107	CAW ions Σ	/G_ of (₪ 1	C4YQN7 Score 7.12	Hits 6	Correlation 0.937	1661.866	-0.254	52.1	2	Tag	1.07E+05	2	t:
Protein: C4YQI ptide ion views Pi Conflicting prot Accession (P43098 • C4YQN7 (+1)	N7 Fat rotein reso teins for Peptides 42 41	ty acid olution r peptid Unique 3 2	e ion 10 Conflict: 48	e alpha sub 7 Protein Score 345	unit red Pe ☑ 7.12	P I 2 2 3	eptide # [] 107 [] 8671	ions Σ	/G_ of (□ 1 2	C4YQN7 Score 7.12 	Hits 6 	Correlation 0.937 0.146	1661.866 1507.822	-0.254 5.75	52.1 40.2	2		1.07E+05 2.47E+04	2	t:
Protein: C4YQI ptide ion views Pi Conflicting prot Accession (P43098 • C4YQN7 (+1)	N7 Fat rotein reso teins for Peptides 42 41	ty acid olution r peptid Unique 3 2	e ion 10 Conflict: 48	e alpha sub 7 Protein Score 345	unit red Pe ☑ 7.12	P I 2 3 4 5 5	eptide # 107 8671 2379	ions Σ	/G_ of (□ 1 2 0	C4YQN7 Score 7.12 8.05	Hits 6 8	Correlation 0.937 0.146 0.881	1661.866 1507.822 1145.601	-0.254 5.75 7.45	52.1 40.2 48.7	2 4 2 2		1.07E+05 2.47E+04 4.99E+04	2	t:

Resolution of conflicts for this protein

Accession	Peptides	Unique	Conflict	Score	Tag	* ^		#	Σ	④ Sco	re Hit	ts Correlation	n Mass	Mass error (p	RT (mins)	Charg	je Tag	 Abundance 	Conflict
P34731	58	58	0	529				107		1 7	12 6	0.932	1661.866	-0.254	52.1	2		1.07E+05	1
🔇 C4YQR7	0	0	0	A				8671	0	2 -		- 0.093	1507.822	175 B	40.2	4		2.47E+04	1
C4YQN7 (+1)	0	0	0	0			V	2379	0	0 8	05 8	0.910	1145.601	<u> 45</u>	48.7	2		4.99E+04	0
🌍 P43098 🛛 🤇	4 2	40	2	345			\checkmark	2779	٠	2 7	49 9	0.866	1277.678	9.58	57.4	2		5.31E+04	0
P41797	27	11	43	314		-	1	2930	0	0 8	13 9	0.937	1414.678	3.49	46.2	2		4.09E+04	0
•	III					•	•	_				III							+
Drotoin: D420(98 Fatty	acid s	ynthase	subunit alp	ha GN:	=FAS2	2 PE=	3 SV	-1										
FIDIEIN: P430:																			
 Protein: P430 Protein: Q5A0 		saldola	ase GN=	TAL1PE=	<u>S</u> V=1									-					
	17 Tran		ase GN=											Г	_				
Protein: Q5A0	17 Tran	ution		F			eptid	e ion	s of	Q5A017					F				
Protein: Q5A0 Peptide ion views Conflicting pro	17 Tran Protein resol	ution peptide	e ion 10	F			eptid #	e ion Σ		-		Correlation	Mass N	Aass error (p	F RT (mins)	Charge	Tag	✓ Abundance	Conflict:
Protein: Q5A0 Peptide ion views Conflicting pro	17 Tran Protein resol	ution peptide	e ion 10 Conflict:	7 E	F	P	#	Σ		-			Mass N 1661.866	Aass error (p 8.95	· .	Charge 2	Tag	Abundance 1.07E+05	Conflict:
Protein: Q5A0 Peptide ion views P Conflicting pro Accession	17 Tran Protein resol Deteins for Peptides	ution peptide Inique	e ion 10 Conflict:	7 Protein Score	F V 7.	Pe; .12	#	Σ 17 0	@ 1	Score	Hits	0.979			RT (mins)	-	Tag		
Protein: Q5A0 Peptide ion views P Conflicting pro Accession P43098	17 Tran Protein resol teins for Peptides U 42	ution peptide Inique 40	e ion 10 Conflict: 2	7 Protein Score	F 7.	Per .12	#	Σ 17 · ·	@ 1	Score 8.69	Hits 9	0.979 0.957	1661.866	8.95	RT (mins) 52.1	2	Tag	1.07E+05	1 🧃 🗉
Protein: Q5A0 Peptide ion views P Conflicting pro Accession P43098 C4YQN7 (+1)	17 Tran Protein resol oteins for Peptides L 42 0	ution peptide Inique 40 0	e ion 10 Conflict: 2	7 Protein Score	F 7.	Pe; .12 .12 .69	# ✓ 10 ✓ 52	Σ 17 0 10 •	中 1 0 0	Score 8.69 8.45	Hits 9 8	0.979 0.957 0.968	1661.866 1345.749	8.95 29.6	RT (mins) 52.1 67.6	2	Tag	1.07E+05 1.62E+05	1 🧯 🗉 0 🐧
Protein: Q5A0 Peptide ion views P Conflicting pro Accession P43098 C4YQN7 (+1)	17 Tran Protein resol oteins for Peptides L 42 0	ution peptide Inique 40 0	e ion 10 Conflict: 2	7 Protein Score	F 7.	Pe; 112	<i>#</i> ✓ 10 ✓ 52 ✓ 53	Σ 17 0 10 • 13 • 14 0	1 0 0	Score 8.69 8.45 8.77	Hits 9 8 9	0.979 0.957 0.968 0.957	1661.866 1345.749 1880.959	8.95 29.6 11.7	RT (mins) 52.1 67.6 52.9	2 2 2	Tag	1.07E+05 1.62E+05 9.65E+04	1 (= 0 () 0 ()

Finally move to the remaining conflict in panel B, and favour the higher scoring protein

ccession	Peptides	s Unique	Conflic	t: Score	Tag	•		#	Σ	由 Sco	re Hi	its Correlatio	n Mass	Mass error (p.	RT (mins)	Charg	je Tag	 Abundance 	e Confl
P34731	58	58	0	529				107	٠	1 7	12 6	5 0.932	1661.866	-0.254	52.1	2		1.07E+05	0
C4YQR7	0	0	0	0			V	8671	0	2 -		0.093	1507.822	5.75	40.2	4		2.47E+04	1
C4YQN7 (+1)	0	0	0	0			V	2379	0	0 8	05 8	8 0.910	1145.601	7.45	48.7	2		4.99E+04	0
P43098 o	42	41	1	345			V	2779	0	2 7.	49 9	9 0.866	1277.678	9.58	57.4	2		5.31E+04	0
P41797	27	11	43	314		-	\checkmark	2930	0	0 8	13 9	9 0.937	1414.678	3.49	46.2	2		4.09E+04	0
					•	•	4												
Protein: P4309 Protein: Q5A50 tide ion views Protein: Pro	Q8 40S	S riboso	mal pro	tein S4 GN=		PE=4	SV=	1	sof	054508									
Protein: Q5A50 tide ion views Protonflicting prot	28 409 rotein resi teins fo	i riboso olution r peptid	mal pro	tein S4 GN=	RPS4A	PE=4	sv =	=1 e ion:		Q5A5Q8		Correlation	Marc	Macc error (n	PT (minc)	Charge	Tag	- Abundance	Conflicts
Protein: Q5A50 tide ion views Pr Conflicting prot Accession	28 409 rotein rese teins fo Peptides	riboso olution r peptid Unique	mal pro le ion 86 Conflict:	tein S4 GN= 571 Protein Score	RPS4A Pe	PE=4	eptid	e ions	₽	Score	Hits				RT (mins)	Charge	Tag	✓ Abundance 2.475 • 04	Conflict:
Protein: Q5A50 tide ion views Pi Conflicting prot Accession P43098 •	Q8 40S rotein rese teins fo Peptides 42	S riboso olution r peptid Unique 41	mal pro le ion 86 Conflict:	tein S4 GN= 571 Protein Score 345	•RPS4A ₽e 	PE=4	eptid #	e ion: Σ	@ 2	Score	Hits	0.316	1507.822	29	40.2	4	Tag	2.47E+04	1 (
Protein: Q5A50 stide ion views Protection of the	28 40S rotein rese teins for Peptides 42 13	s riboso olution r peptid Unique 41 5	mal pro le ion 86 Conflict: 1	tein S4 GN= 571 Protein Score 345 116	Pe	PE=4	eptid # 867 7 480	= 1 e ions Σ 71 0 04 0	2 1	Score 7.95	Hits 2	0.316 0.896	1507.822 831.466	29 2.55	40.2 33.9	4 2	Tag	2.47E+04 1.76E+04	1 (
Protein: Q5A50 stide ion views Protection of the	Q8 40S rotein rese teins fo Peptides 42	S riboso olution r peptid Unique 41	mal pro le ion 86 Conflict: 1	tein S4 GN= 571 Protein Score 345	•RPS4A ₽e 	PE=4	eptid # 867 867 867 146	e ions Σ 71 0 04 0	₽ 2 1 3	Score 7.95 8.23	Hits 2 8	0.316 0.896 0.552	1507.822 831.466 980.493	29 2.55 11.7	40.2 33.9 28.5	4	Tag	2.47E+04 1.76E+04 1.36E+05	1 0
Protein: Q5A50 htide ion views Protonflicting proton Accession	28 40S rotein rese teins for Peptides 42 13	s riboso olution r peptid Unique 41 5	mal pro le ion 86 Conflict: 1	tein S4 GN= 571 Protein Score 345 116	Pe	PE=4	eptid # 7 867 7 480 7 146 7 188	e ions Σ 71 0 04 0 51 • 17 0	2 1 3 0	Score 7.95 8.23 7.48	Hits 2 8 6	0.316 0.896 0.552 -0.061	1507.822 831.466 980.493 1507.782	29 2.55 11.7 2.57	40.2 33.9 28.5 40.2	4 2	Tag	2.47E+04 1.76E+04 1.36E+05 6.24E+03	1 0 0 0
Protein: Q5A50 stide ion views Protection of the	28 409 rotein reso teins fo Peptides 42 13 0	s riboso olution r peptid Unique 41 5	mal pro le ion 86 Conflict: 1	tein S4 GN= 571 Protein Score 345 116	Pe	PE=4	eptid # 867 867 867 146	e ions Σ 71 0 04 0 51 • 17 0	2 1 3 0	Score 7.95 8.23	Hits 2 8	0.316 0.896 0.552	1507.822 831.466 980.493	29 2.55 11.7	40.2 33.9 28.5	4 2	Tag	2.47E+04 1.76E+04 1.36E+05	1 0

Adopting a similar approach to the next protein favouring the protein with the highest score as each conflict is examined.

Accession	Peptic	les Uniqu	e Conflic	ct: Score	Tag	-	-		Σ	色 Scor	e Hi	its Correlation	n Mass	Mass error (p.	RT (mins)	Charge	Tag	 Abundance 	e Co	nflict ^
C4YQR7	0	0	0	0			V 2	2054		0 8.4	14 (5 0.976	1674.74	10.1	33.2	2		4.21E+04		4
C4YQN7 (+1)	0	0	0	0	•		V 1	2391	0	1 7.9	96 9	0.863	1525.747	8.3	57.5	3		1.36E+04		3
P43098	42	42	0	345			V 1	4084	0	0 7.	6 3	0.910	1606.749	5.3	40.5	2		1.68E+04		3
P41797	o 27	11	43	314			V	182	0	1 8.4	14 :	0.914	1674.76	21.6	33.2	3		8.15E+04		3
P46587	34	18	41	372	<u>_</u>	۸-	V 1	7705	0	0 8.	6 !	5 0.608	1786.989	3.48	52.3	B ²		4.37E+03	\mathbf{v}	3 -
•	II.	1					•									D				+
Protein: P41	797 He	at shoc	k proteir	n SSA1 GN=	SSA1 PE	E=1 S	V=2													
FIOLEIII. F41																				
	587 He	at shoc	k protein	SSA2 GN=	SSA2 PE	E=1 S\	V=3													
Protein: P46			k proteir	SSA2 GN=	SSA2 PI	E=1 S\	V= 3													
	Protein re	esolution			SSA2 PI			ions	of F	46587							7			
Protein: P46 eptide ion views	Protein re roteins f	esolution for pepti	ide ion 2		SSA2 PE				_	A6587 Score	Hits	Correlation	Mass 1	Mass error (p	RT (mins)	Charge - Ta	aq 🗸	Abundance	Conflic	t:
Protein: P46 ptide ion views Conflicting pr	Protein re roteins f	esolution for pepti	ide ion 2 Conflict:	054			eptide		_		Hits 6		Mass 1 1674.74	Mass error (p 10.1	RT (mins) 33.2	Charge Ta	aç 🗸	Abundance	Conflic 4	t:
Protein: P46 eptide ion views Conflicting pr Accession	Protein re roteins f Peptides	esolution for pepti Unique	ide ion 2 Conflict: 41	054 Protein Score	P	Ē	eptide	Σ	啩	Score		0.946				Charge FTa	ag 🗸			tt 🏠
Protein: P46 eptide ion views Conflicting pr Accession P46587	Protein re roteins f Peptides 34	esolution for pepti Unique 18	ide ion 2 Conflict: 41	054 Protein Score 372	P ∕ ▼ 8.		eptide # 2054	Σ 0	啩	Score 8.44	6	0.946 0.804	1674.74	10.1	33.2	b 🗧	ag -	4.21E+04	4	tt: G
Protein: P46 eptide ion views Conflicting pr Accession P46587 P41797	Protein re roteins f Peptides 34 27	esolution for pepti Unique 18	ide ion 2 Conflict: 41 = 43 = 37 1	054 Protein Score 372 314	₽ × ▼ 8.		2054	Σ 0 1 0 4 0	日 0 1	Score 8.44 7.96	6 9	0.946 0.804 0.912	1674.74 1525.747	10.1 8.3	33.2 57.5	3	ad 🗸	4.21E+04 1.36E+04	4 3	tt ^
Protein: P46 eptide ion views Conflicting pu Accession P46587 P41797 P10591	Protein re roteins f Peptides 34 27 15	esolution for pepti Unique 18	ide ion 2 Conflict: 41 : 43 : 37 1 7 (054 Protein Score 372 314 153	P ² ▼ 8. ▼ 8. ▼ 8.		# 2054 1239: 1408-	Σ 0 1 0 4 0 0	0 1 0 1	Score 8.44 7.96 7.76	6 9 3	0.946 0.804 0.912 0.971	1674.74 1525.747 1606.749	10.1 8.3 5.3	33.2 57.5 40.5	3 2	ag 🗸	4.21E+04 1.36E+04 1.68E+04	4 3 3	

In this case the first peptide for protein (P41797) has 4 conflicting proteins in panel E. Resolve the conflict in favour of the protein with the higher score (P46587) by unticking the peptide ion in panel B then move on to the next conflicting peptide ion in Panel B (which has 3 conflicts) and resolve the conflict in favour of the protein with the higher score.

Accession	Рер	tides Uniqu	e Conflict	Score	Tag 🔻	*	*		Σ	き Scor	e Hi	ts Correlatio	on Mass	Mass error (p	RT (mins) Char	ge Tag	 Abundance 	e Confl	ict ^
C4YQR7	C	0	0	0			V 2	054	0	D 8.4	44 6	0.976	1674.7	4 10.1	33.2	2		4.21E+04	1	Ľ
🚳 C4YQN7 (+:	L) (0	0	0			V 12	2391	0	1 7.9	96 9	0.863	1525.74	47 8.3	57.5	3		1.36E+04	2	T.
P43098	4.	2 42	0	345			V 14	1084	0	0 7.	76 3	0.910	1606.74	49 5.3	40.5	2		1.68E+04	2	
P41797	<u> </u>	12	27	314			V 1	182	0	1 8.4	44 5	0.914	1674.7	6 21.6	33.2	3		8.15E+04	2	
P46587	3	18	26	364		-	V 17	7705	0	0 8.	6 5	0.608	1786.98	89 3.48	52.3	2		4.37E+03	2	-
٠		111			+		٠ 📃													F.
Protein: P			k protein	SSA2 OS≕	Saccharo	myc	es cer	evis	iae (GN=SS	A2 PE=	1 SV=3								
Peptide ion view	s Protein	resolution																		
Conflicting	proteins	for pept	ide ion 20	54		Pe	eptide	ions	of P	10592										
Accession	Peptid	s Unique	Conflict: P	rotein Score	P ^		#	Σ	囤	Score	Hits	Correlation	Mass	Mass error (p	RT (mins)	Charge	Tag	✓ Abundance	Conflict:	•
P46587	34	18	26 36	54	8.4		2054	•	0	8.18	3	0.940	1674.74	10.1	33.2	2		4.21E+04	1 (6
P41797	o 27	12	27 31	4	🗸 8.4 🚍		/ 8166	0	4	7.21	2	0.896	960.52	7.04	21.2	2		1.18E+04	1 (6
P10591	0	0	0 0		8.		34780	•	0			0.928	960.535	23.3	21.2	3	-	1.67E+03	0 (١.
O5A3Y2	8	7	2 6	3.6	5.0		/ 18915	•	0	6.32	3	0.621	1423.668	7.06	18.2	3		4.16E+03	0 ([
P10592	7		off all pepti	-	🗸 8. 👻		44755	•	0	6.32	3	0.570	1423.667	6.58	18.2	4		365	0 (<u>-</u> ک

Alternatively you can review the other conflicting proteins. In this case P10591 and P10592 are from a similarly named protein but a different species so you can right click and turn off all the peptides.

Then reviewing the remaining conflicts between P41797 and P46587 they have 19 remaining conflicts. To resolve these in favour of P46587 you can highlight all the remaining conflicted peptides in panel B and click on the tick box to untick them, this will remove them from P41797.

Accession	Peptide	s Unique	Conflic	t: Score	Tag	* ^		#	Σ	b	Score I	Hits Correlat	ion Mass	Mass error (p	RT (mins)	Charg	e Tag	 Abundance 	e Cor	nflict
C4YQR7	0	0	0	0				1400		1	8.63	8 0.970	2166.04	6 13.8	59.7	3		7.11E+04	1	1
P43098	42	42	0	339			v	690		1	8.58	8 0.822	1283.70	6 11.4	68.8	2		1.09E+05	1	1
C4YQN7 (+1)	0	0	0	0				297	0	1	8.06	4 0.913	1680.83	8 16	52.8	2		1.61E+05	1	1
P41797	o 27	12	19	314			V	179	٠	3	8.46	9 0.634	1472.79	8 9.86	49.2	2		9.33E+04	0	0
P46587	34	19	20	373		-		6149	0	1	7.93	9 0.977	1230.69	7 -0.111	35.4	3		1.95E+04	c	0
						P-	4													•
Protein: P46 ptide ion views			protein	SSA2 GN=	SSA2 PI	E=1 \$	SV=3													
	Protein res	olution	· .		SSA2 PI		S V=3 Peptid	le ior	ns of	P465	87									
tide ion views	Protein res	olution	te ion 29		SSA2 PI			le ior Σ	ns of			Correlation	Mass	Mass error (p	RT (mins)	Charge	Tag	 Abundance 	Conflict	t
tide ion views	Protein res roteins fo	olution	le ion 29 Conflict:	97		pt	Peptid	Σ	_		re Hits	Correlation 0.961	Mass 1680.838	Mass error (p 16	RT (mins) 52.8	Charge 2	Tag	 Abundance 1.61E+05 	Conflict 1	t:
tide ion views Conflicting pr Accession	Protein res roteins fo Peptides 34	olution or peptio Unique	le ion 29 Conflict: 20 3	97 Protein Score	Pe	pt 5	Peptid #	Σ 97 Φ	· @	Scor 8.	re Hits						Tag		Conflict 1 1	t: G
tide ion views Conflicting pr Accession P46587	Protein res roteins fo Peptides 34	olution or peptio Unique 19	le ion 29 Conflict: 20 3	97 Protein Score 73	Pe	pt 5	Peptid #	Σ 97 0 364 0) (P) (P) (P) (P) (P) (P) (P) (P) (P) (P)	Scor 8.	re Hits 06 4 63 8	0.961	1680.838	16	52.8	2	Tag	1.61E+05	Conflict 1 1 1	t: G
tide ion views Conflicting pr Accession P46587	Protein res roteins fo Peptides 34	olution or peptio Unique 19	le ion 29 Conflict: 20 3	97 Protein Score 73	Pe	pt 5	Peptid # 29	Σ 97 0 364 0 32 0) (P) (P) (P) (P) (P) (P) (P) (P) (P) (P)	Scor 8.0 8.0	re Hits 06 4 63 8 59 7	0.961 0.703	1680.838 2166.023	16 2.92	52.8 59.7	2	Tag	1.61E+05 3.02E+03	Conflict 1 1 1 1	t:
tide ion views Conflicting pr Accession P46587	Protein res roteins fo Peptides 34	olution or peptio Unique 19	le ion 29 Conflict: 20 3	97 Protein Score 73	Pe	pt [5	Peptid # 29 303	Σ 97 0 364 0 32 0 19 0		Scor 8.0 8.0 8.0	re Hits 06 4 63 8 59 7 64 8	0.961 0.703 0.921	1680.838 2166.023 1590.767	16 2.92 13	52.8 59.7 45.8	2 2 2	Tag	1.61E+05 3.02E+03 7.77E+04	Conflict 1 1 1 1	t: 6

The display will update to show one remaining conflict for P46587. Click on this protein in panel A.

Accession	Peptid	es Unique	Conflic	t: Score	Tag	▼ ^		#	Σ	中 So	ore H	lits Correlatio	on Mass	Mass error (p	RT (mins)	Charg	ge Tag	 Abundance 	e Con	mic
C4YQR7	0	0	0	0				186		7	6.58	2 0.959	1292.682	1.38	49.9	2		3.01E+05	1	
C4YQN7 (+1)	0	0	0	0	•		V	1182	0	1	8.44	5 0.975	1674.76	21.6	33.2	3		8.15E+04	0)
P43098	42	42	0	345			1	155	•	3	8.48	8 0.968	1658.914	15.7	56.3	2		1.99E+05	0)
P41797	12	12	0	144				3054	•	1	8.48	7 0.983	1658.916	16.8	56.3	3		5.3E+04	0)
P46587 (o 34	33	1	363		-	V	184	•	3	8.39	6 0.985	1470.725	17.9	52.7	2		2.15E+05	0)
						P.						III								
Protein: P4658 Protein: Q5AE otide ion views	OM7 G Protein re	lycerald solution	ehyde 3	-phosphate		ogen	ase													
Protein: Q5AD	OM7 G Protein re	lycerald solution	ehyde 3	-phosphate		ogen	ase			3 PE=3 Q5AD/										
Protein: Q5AE tide ion views F onflicting pro	OM7 G Protein re	lycerald solution or peptic	e hyde 3 e ion 18	-phosphate		ogen P	ase		s of	Q5AD/	47	Correlation	Mass	Mass error (p	RT (mins)	Charge	Tag	 Abundance 	Conflict	t
rotein: Q5AC ide ion views F onflicting pro Accession F	OM7 G Protein re	lycerald solution or peptic	e ion 18	-phosphate	e dehydr	ogen P ot	ase (eptic	le ion Σ	s of	Q5AD/	N7 Hits		Mass 1 1292.682	Mass error (p	RT (mins) 49.9	Charge 2	Tag	 Abundance 3.01E+05 	Conflict 1	te
Protein: Q5AC tide ion views F onflicting pro Accession F P46587 •	DM7 G Protein re Dteins f Peptides	lycerald solution or peptic	e ion 18 Conflict: 1	- phosphate 16 Protein Score	e dehydr Pep	ogen P ot	ase eptic #	le ion Σ 36 ○	s of @	Q5AD/ Score	M7 Hits 5 5					Charge 2 2	Tag			t
rotein: Q5AC ide ion views F onflicting pro Accession F P46587 •	DM7 G Protein re Dteins fr Peptides 34	lycerald solution or peptic Unique	e ion 18 Conflict: 1	-phosphate 16 Protein Score 63	e dehydr Per Ø 6.58	P pt	eptic #	le ion Σ 36 00 ●	s of @ 7 0	Q5AD/ Score 8.45	M7 Hits 5 5 2 9	0.884	1292.682	15.8	49.9	2	Tag	3.01E+05	1	te
Protein: Q5AC tide ion views F onflicting pro Accession F P46587 •	DM7 G Protein re Dteins fr Peptides 34	lycerald solution or peptic Unique	e ion 18 Conflict: 1	-phosphate 16 Protein Score 63	e dehydr Per Ø 6.58	P pt	ase (eptic # 18	le ion Σ 36 0 00 • 5 0	s of 但 7 0	Q5AD/ Score 8.45 9.12	M7 Hits 5 5 2 9 2 8	0.884 0.873	1292.682 2885.364	15.8 5.15	49.9 76.6	2 2	Tag	3.01E+05 4.07E+04	1 0	t
Protein: Q5AD tide ion views F onflicting pro Accession F	DM7 G Protein re Dteins fr Peptides 34	lycerald solution or peptic Unique	e ion 18 Conflict: 1	-phosphate 16 Protein Score 63	e dehydr Per Ø 6.58	P P t t	ase (# 7 18 7 32 7 (7 18	le ion Σ 36 0 00 • 5 0	s of ⊕ 7 0 0 0	Q5AD/ Score 8.45 9.12 8.02	M7 Hits 5 5 2 9 2 8 2 7	0.884 0.873 0.969 0.757	1292.682 2885.364 2889.489	15.8 5.15 1.97	49.9 76.6 63.9	2 2	Tag	3.01E+05 4.07E+04 2.4E+05	1 0 0	te

The strategies that can be used to resolve conflicts can include differences in Mass error as well. However, if there is no difference for a given peptide then either resolution in favour of the protein with the highest protein score or unticking the peptide for both conflicting proteins is an option.

Note: the abundances will need to be recalculated as a result of performing Conflict resolution. This is achieved by clicking on the Recalculate abundances, which appears during Conflict resolution

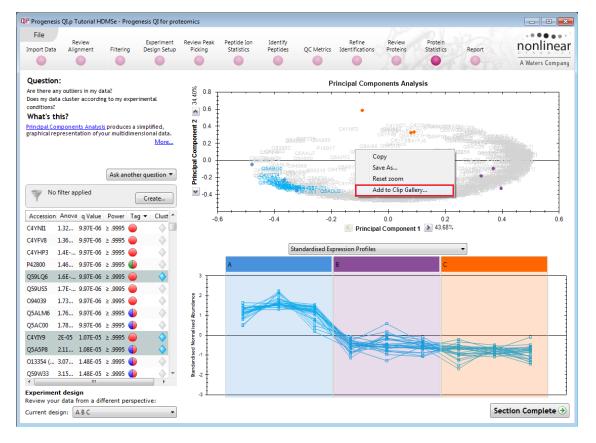
< III		4	•
• Refine Identifications	Protein options	Recalculate a	bundances

Appendix 8: Using Clip Gallery to Save and Export Pictures and Data

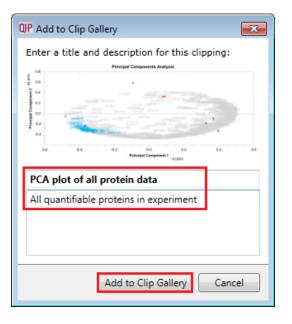
At every stage of the Progenesis QI for proteomics workflow the views and data tables can be added to the Clip Gallery.

The saved images of the Views and the tables are retained as part of the experiment and are stored accordingly. This facility allows you to capture (high resolution) images that can be used in the development of specific reports and/or used as part of the process of publishing your experimental findings.

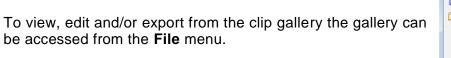
As an example of using the Clip Gallery, at the **Protein Statistics** view while displaying the PCA plot right click on the **Biplot** View and select **Add to Clip Gallery**...

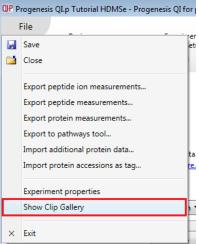


This will open a dialog displaying what is to be saved and allows you to alter the title and provides a description of the image for later reference.



Enter details as required and click Add to Clip Gallery





Selecting an image in the gallery makes available an **Actions** menu that allows you to manipulate the output of the image.

QIP Clip Gallery		
Clip Gallery	Item si	ze: D Search
Actions Edit Edit Copy Imag Copy Title Copy Desc Export Ima Delete	ription	Data analysis performed using: Progenesis QI For proteomics www.nonlinear.com
PCA plot of all protein data All quantifiable proteins in experiment	Progenesis QI for proteomics logo A high-resolution image that you can use in presentations and posters to show the software was used in your research.	Progenesis QI for proteomics analysis badge A high-resolution image that you can use in presentations and posters to show the software was used in your research.
		Export all Close

Note: there is also the capacity to **Export all...** the images in the experiments clip gallery which creates a list of files in a folder of your choice where the file name is based on the image title.

Note: right clicking on a table and adding it to the Clip Gallery allows you to export the current content to Excel.

Appendix 9: Exporting protein identities to IPA

IPA (Ingenuity Pathway Analysis): is a commercial web-based software application for the analysis, integration and interpretation of data derived from any experiments that generate gene and chemical lists with expression data, such as metabolomics and proteomics.

Before exporting data to IPA, make sure that you can launch IPA using the default browser on your system. The plug-in relies on the browser to upload the Progenesis-exported data to IPA.

Note: the use of IPA requires a licensed copy, for licensing details please visit the IPA website.

First select the Protein data to export to the pathways tool using tag filtering to 'focus' the set to export.

Click Export to pathways tool and select IPA and click Next.

QIP Progenesis QI.p Tutorial HDMSe - Progenesis QI for prot	eomics	
File	leview Peak Peptide Ion Identify Refine Review Protein Picking Statistics Peptides QC Metrics Identifications Proteins Statistics	Report A Waters Company
<text><text><text><text><text><text><text><text><text><text></text></text></text></text></text></text></text></text></text></text>	No filter applied Create Search Accession Peptides Unique peptides Confidence score Anova (p) Tag V Max f OP Export Pathways Information Select a pathways tool Choose a pathways tool from the list below. You can find out more or download new plugins using the links below. Which pathways tool do you want to use? IPA: Ingenuity Pathway Analysis About this plugin & Download other plugins	A Waters Company Welp Old change Highest Mean Lowest B C B A C B A C A C B A C A C B A C A C
View peptide measurements You can also double-click to review a protein. 4 Export data for further processing By exporting your data to external tools, there's no limit to your analysis.		B A B A SV=1
Export to pathways tool Export protein measurements Export peptide measurements Export peptide ion measurements	< Back Next > Cancel	C I∳I
Experiment design Review your data from a different perspective: Current design: A B C	∢ Quantifiable proteins displayed: 512	Section Complete 🤿

Progenesis QI for Proteomics User Guide

Select the appropriate identifier type used for proteins in the experiment.

Note: for proteins with different identifier types, filter the protein list to contain only compounds with single identifier type at a time, then perform the export operation and merge the resulting lists in IPA.

Finally select the way you want to export the compounds to IPA.

QIP Export Pathways Information
Configure your export Choose which identifications to export and the type of analysis you want to perform.
Select the identifier type used for proteins in the experiment:
UniProt/Swiss-Prot Accession 🔹
UniProt/Swiss-Prot Accession
GenPept GI Number
For expression data, choose two experimental conditions that you would like to compare.
Baseline: Comparison:
🗖 A 🔹 🖛 🖛
If greater, gives a negative fold change. If greater, gives a positive fold change.
< Back Export proteins to IPA Close

If you are performing over-representation analysis or other types of analysis that do not require the protein expression data then you can make use of the **Create a list** option to export data to IPA.

If you require to perform enrichment analysis or other types of analyses that use protein expression data, select the option to **Upload expression dataset** to IPA.

OPP Export Pathways Information
Configure your export Choose which identifications to export and the type of analysis you want to perform.
Select the identifier type used for proteins in the experiment:
UniProt/Swiss-Prot Accession 🔹
Select the way you want to export the proteins to IPA:
Upload expression dataset
For expression data, choose two experimental conditions that you would like to compare. Baseline: A Comparison: A C A If greater, gives a negative fold change. C A B C C
< Back Export proteins to IPA Close

Note: You need to select the type of protein identifier used by at least one protein whose expression data you want to export. If there are proteins with different identifier types, you will be able to select additional identifier types in IPA after the dataset is uploaded.

The operation exports data from a single observation at a time - select the two experimental conditions (taken from the current **experiment design as set in Progenesis QI for proteomics** you wish to compare as that observation).

Click Export compounds to IPA.

ngenuity – Ingen	uity Login × +	
→ C 🔒	apps.ingenuity.com/ingsso/login?service=https%3A%2F9	62Fanalysis.ingenuity.com%2Fpa%2Fj_spring_cas_security_ch 🖈 🤇
QIAGEN —	lesse login	Contact QIAGEN
Email Password	smith@work.com Remember my password LOGIN	Email Customer Support and Sales ts-bioinformatics@qiagen.com BioinformaticsSales@qiagen.com Phone Customer Support and Sales US Toll Free: +1 866 464 3684 Denmark Toll Free: +45 80 82 0167
	Find out more Forgot Password	Additional Global Phone Numbers Customer Support: +1 (650) 381-5111 Sales: +1 (650) 381-5056 Hours: 00:00 - 16:00 PST (09:00 - 00:00 CET) Monday - Friday (excluding holidays)

If you have access to a licenced copy of IPA then log in

IPA will open displaying the imported data from Progenesis QI for Proteomics.

M IPA											×
<u>F</u> ile <u>E</u> dit	t <u>V</u> iew <u>W</u> indow	<u>H</u> elp							Provide Feedback Support	Janucz Nykiel Clo	se IPA
			Genes and Chemicals	Diseases and	Functions Path	ways and Tox Lists					
NEW ¥			inter gene names/sym			-			RCH Advanced Search		
NEW V		Ľ	inter gene names/syn	100IS/105 of chem	ical/drug names nere			<u>S</u> E4	Advanced Search	Q	IAGEN
Dataset U	Ipload - New Dataset	2016-05-20 10:29	AM						Dataset Upload Workflow Instructions		
1. Select	File Format:	Flexible	Format	•	More Info						
2. Conta	ins Column Header:	O Yes	No						Data Upload Workflow	N	
2 Coloct	Identifier Type:	UniDrot	/Swiss-Prot Accession		Specify the identifie	r type found in the data	t		Use Dataset Upload to import your dataset		
				·					Once uploaded, many different analysis opt Biomarker Filter, Molecular Tox and Core Ar		
4. Array	platform used for exp	eriments: Not sp	ecified/applicable	•	Select relevant array	platform as a reference	e set for dat	a analysis.	the different type of analyses and see whi		
5. Use th	ne dropdown menus to	specify the colum	n names that contain	identifiers and ob	servations. For obser	vations, select the appr	opriate exp	ression value ty	p your needs.		
									1. To upload a dataset file, click here.		
Raw Da	ata (512) 🛛 Dataset Sun	nmary (1) 🔪									
	I	[* Open	<u> </u>	
	ID 👻	Observation 1	▼ Ignore	▼ Ignore	▼ Ignore	▼ Ignore	•	Ignore	Look jn: 🗀 Multple Rank. 👻	🐿 🚵 🍱 🔡 BB B=	
		Exp Fold Chan	-						Batch (mult-timepoint) Afry_with_p-value_and_fold.txt		
1	P34731	-1.0090215538908	7				-		LL_with_Fold and_Normalized.txt		
2	C4YQR7	1.03618874042727									
3	C4YMC3	-1.1630436093987									
4	C4YQN7	-1.1424808855243	7								
5	P43098	-1.0016726083821	5								
6	C4YR46	1.11144195146305									
7	O13430	1.0005161363124							File Name:		
8	P46587	1.16697510036399							Files of Type: All Files	•	
9	P82610	1.14143675382931								Open Cancel	
10	P46598	1.06004923965961									
11	Q96VB9	-1.1061363692453	5								
12	P41797	1.50747573107542							Select the dataset file from your computing	ter and click the	
13	P28877	-1.2062486205939	8						Open button.		
14	C4YK39	1.05494661189734							D. Colort Fluidely County County Classes	Course the s	
15	Q59KZ1	1.03999813845519							 Select Flexible format for the file format drandown manual 	irom the	
16	C4YL05	-1.0312270752554							dropdown menu.		
17	P46273	1.31165982609058							4. Select an Identifier Type from the drop	down menu TDA	
18	C4YIL8	1.26909493012736							supports many identifiers and symbols and		
19	O94039	1.55325257659893							guess at the type of identifier in your data		
20	013287	1.38535524141575					-		override the selection, uncheck the option		
	•				3333	88			the most appropriate one. If more than on	e type of identifier	
									 exists in your dataset, select all appropriate 	ones.	
									GenBank 💌		

You can now explore your protein expression data using the tools available in IPA. Instructions on how to manage and explore your imported data set are provided by IPA.

Appendix 10: Exporting protein identities to Metacore

Metacore: is a commercial web-based software application for the analysis, integration and interpretation of data derived from any experiments that generate gene and chemical lists with expression data, such as metabolomics and proteomics.

Before exporting data to MetaCore, make sure that you can launch MetaCore using the default browser on your system. The plug-in relies on the browser to upload the Progenesis-exported data to MetaCore.

First select the Protein data to export to the pathways tool using tag filtering to 'focus' the set to export.

Click Export to pathways tool and select MetaCore

Image: Progenesis QI.p Tutorial HDMSe - Progenesis QI for progenesis QI.p Tutorial HDMSe - Progenesis QI for progene	teomics	
File Review Experiment Import Data Alignment Filtering Osign Setup	Review Peak Peptide Ion Identify QC Metrics Identifications Protein Statistics Repo	nonlinear A Waters Company
Review Proteins Using this screen, you can find the proteins of interest in your experiment.	Vo filter applied Create	
 Set the quantitation options If you're not already done so, choose between relative and absolute quantitation, use of Hi-N, protein grouping and more. Protein options Create a shortlist to review In the table, sort and filter the proteins based on their measurements, to generate a shortlist for further review. • How are the measurements calculated? To sort the table by a given value, simply click the relevant column header. 	Accession Peptides Unique peptides Confidence score Anova (p) q Value Tag Max fold DP Export Pathways Information Select a pathways tool Choose a pathways tool from the list below. You can find out more or download new plugins using the links below. Which pathways tool do you want to use? MetaCore About this plugin Download other plugins 	d change Highest Mean Lowest Me. B C A B A B C A B B A C B C A B C A B C A B C A B C A B C A B C A B A C
 3 Review the proteins For each protein of interest, review its peptide measurements and correlations: View peptide measurements You can also double-click to review a protein. 4 Export data for further processing By exporting your data to external tools, there's no limit to your analysis. 	123 119 128 131 E=3 SV=	A C C B C B
Export poptide measurements Export peptide measurements Export peptide ion measurements Export peptide ion measurements	< Back Next > Cancel 3.0 1 2.5 1 1.5 ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	c ×≢r
Experiment design Review your data from a different perspective: Current design: A B C •	4 Quantifiable proteins displayed: 516	Section Complete 🏵

Then click Next to Configure your export page .

Select the appropriate identifier type used for proteins in the experiment.

QP Export Pathways Information
Configure your export Choose which identifications to export and the type of analysis you want to perform.
Select the identifier type used for proteins in the experiment:
Swiss-Prot Accession 🔹
Swiss-Prot Accession
GI Number ENSEMBL EMBL
comparison with the selected control.
< Back Export proteins to MetaCore Close

If you have multiple conditions in your current experiment design you can select which condition should be used as the control

Select the identifie		or proteins i	n the experime	nt:	
Swiss-Prot Accessi	on				
Select the control	condition:				
🗆 A					
A					
B C					
<u> </u>					

Finally click **Export proteins to MetaCore**.

If you have not already done so, you will be prompted to enter your MetaCore *User name* and *Password*; once you have done this, your data will be exported to your MetaCore account, and the MetaCore user interface will open in your default web-browser.

Windows Securit	y 💌
	letaCore our username and password for your MetaCore installation. e logged in, your data will be exported to your user account.
	User name Password
	OK Cancel

Metacore will open displaying the imported data from Progenesis QI for Proteomics.

You can now explore your protein expression data using the tools available in MetaCore. Instructions on how to manage and explore your imported data set are provided by MetaCore.

Appendix 11: Waters Machine Specification

This appendix provides information on the approximate time(s) taken at each stage and the total time taken to analyse a set of 9 (Phase 1) HDMSe runs on a Waters Demo Spec PC.

Machine Spec: LenovoProcessor: Intel® Xeon® CPU E5-1630 v3 @ 3.70GHz 12coreK40c GPU cardRAM: 64.0 GBSystem Type: 64-bit Operating System

File Folder Size: Each file folder (.RAW): 40.9 Gig

Analysis Stages:		Per file	Total	
Import Data:	Loading of Raw data per file	10min	1hr 12min	for 9 files
	Apex Background processing	18min (max)	2hr 42min	for 9 files
	(re-opening at Import Data)		20s	
Alignment:	Automatic alignment of data		3min 45s	
	(re-opening at Alignment)		10s	
Peak Detection:	Automatic Detection of data		9min 25s	
	(re-opening at Peak Detection)		10s	
Identify Peptides:	Performing MS ^E Search		13min 15s	
	(re-opening at Identify Peptides)		10s	
Total Analysis Time:	Excluding Background Apex Processing	9	1hr 38min	
	Including Apex processing		2hr 51min	
Restoring:	Tutorial Archive		3.5min	

References

Silva, JC, Gorenstein, MV, Li, G-Z, Vissers, JPC and Geromanos. Molecular and Cellular Proteomics (2006); 5 : 144-156 Absolute Quantification of Proteins by LCMS^E